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RESEARCH ARTICLE

CHROMATOGRAPHIC IDENTIFICATION AND SPECTROPHOTOMETRIC DETERMINATION OF A DIURETIC ALKALOID IN THE ROOTS OF *CATHARANTUS ROSEUS* (APOCYNACEAE) AN ANTIHYPERTENSIVE PLANT USED IN BENIN

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Abstract

Arterial hypertension (AHT) remains a major public health problem because for its high frequency and its cardiovascular complications. Catharantus roseus (L.) G. Don (Apocynaceae) is one of the plants used in Benin for the traditional treatment of hypertension.The general objective of this work is to contribute to the management of arterial hypertension by identifying and determining the active principle of furosemide from the extract of the roots of *Catharantus roseus* (L.) G. Don.The extract was prepared using the method of maceration with distilled water in the proportions 1 m/10v. The extract obtained was used for the extraction of total alkaloids in basic medium with an aprotic polar solvent such as dichloromethane. Furosemide, one of the diuretics frequently used in the clinical center, was identified in the alkaloid extract by the method of analytical thin layer chromatography. The determination of the active principle of furosemide was made by UV-vis spectrophotometry at 276 nm. The yield of the crude aqueous extraction was 12.57%. Thin layer chromatography of the extract showed a spot at the same Rf as that of furosemide which is an alkaloid. The content of furosemide in the extract is 1.27 ± 0.03 µg/g of extract.

Introduction:-

Arterial hypertension (AHT) is a major public health problem due to its high frequency and its cardiovascular complications. More than a quarter (26.4%) of the adult world population is hypertensive, and this proportion should reach at 29.2% by 2025, i.e. nearly 1.6 billion subjects (Fourcade et al. 2007). According to health statistics from the

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World Health Organization (WHO), one in three adults in the world has hypertension. It is believed to be the condition responsible for nearly half of deaths from stroke and heart disease (WHO, 2012).

In France, the prevalence of hypertension is estimated at 30% (Perrine et al. 2018). On the other hand, in Africa, more than 40% of adults in many countries are hypertensive (WHO, 2013). In Benin, its prevalence was estimated at 27.5% (WHO, 2013). As part of the treatment of these hypertensive patients, diuretics represent the most prescribed class of drugs (Blacher et al. 2013). Their action by inhibiting the active transport of chlorine in the ascending limb of the loop, thus opposing the reabsorption of sodium and other ions such as potassium. They also have a specific hemodynamic action (pulmonary and venous capillary vasodilation) which plays an important role in the therapeutic effect during acute pulmonary edema (Hausfater, 2015). The recourse to plants is all the more tending when one takes into account the concerns related to the harmful effects of chemical drugs, the search for a healthy life, the shortage and the high cost of health care (Kagnon et al. 2020). The proven efficacy of plants in the treatment of many pathologies has led researchers to look into the pharmacological properties of plants (Kagnon et al. 2020). Thus several plants have been used in the pharmacopoeia against arterial hypertension, including catharantus roseus (Bio et al. 2015). A decoction of the roots of this plant is recommended to treat high blood pressure (Adomou et al. 2012; Kouadio et al. 2016). The purpose of this work is to perform the research to enhance traditional medicine in order to make health care accessible to the poor population. This work generally aims to extract, identify and determine the content of furosemide in the total alkaloid extract of *catharantus roseus* roots.

Material and Methods:

Plant material

The plant material used (Fig.1) is the underground part (root) of *Catharantus roseus* (L.) G. Don harvested on April 2022 in southern Benin, atSeme in the Oueme department. The root was washed with water, drained and then dried in the shade at a temperature of 25 ± 2°C for 14 days. The plant was certified on April 19, 2022 at the National Herbarium of Benin under number YH697/HNB. Then the dry roots were ground using a Retsch grinder (type SM 2000/1430/Upm/Smf) and the powder was used for the extraction.

Figure 1:- The plant of Catharantus roseus and its dry roots.

Methods:-

Identification of furosemide by thin layer chromatography

Thin layer chromatography (TLC) is a techniqueused for the analysis of plant extracts as it offers the advantage of speed, low cost and allows direct visualization of the separation of compounds (Fig. 2). It was used here to see the chromatographic profile of the pharmacological compounds on the plate.

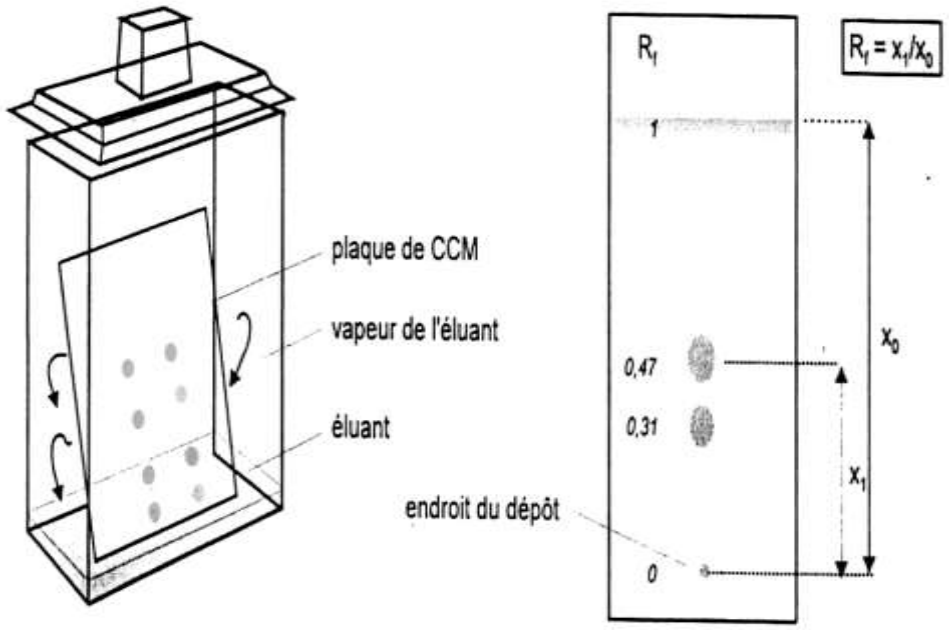


Figure 2:- Picture showing the migration of substances in a CCM tank (Gbaguidi, 2005; Houngbèmè, 2015).

In this work, we applied the chromatographyon normal phase and the detection of the spots of different compounds was done first by direct visualization, then under UV (254 and 366 nm).
The analytical conditions are as follows:

1. Stationary phase: TLC Silica gel 60 F254S Merck®
2. Quantity deposited: 100 µg
3. Mobile phase: toluene-ethyl acetate-formic acid (36-12-5; v/v/v)
4. Detection: UV at 254 nm and 366 nm

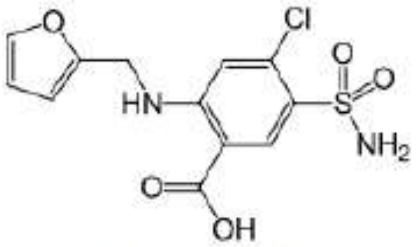
Determination of furosemide by spectrophotometry

Preparation of standards

We prepared a standard stock solution of furosemide (pharmaceutical form) at 1000ppm in distilled water. To do this; one tablet (40mg)of furosemide is dissolved in 40mL of distilled water. Then, by successive dilutions, obtain working solutions at concentrations: 3.12; 6.25; 12.5; 25 and 50ppm which represented the range of concentrations used for calibration.

Extraction of alkaloids

In the litterature; several authors have measured furosemide in its pharmaceutical form by studying quality control parameters (Safila; 2014; Gahandule; 2016; Kalakuko; 2017). During their work furosemide is dissolved and alkalized by a base (1N sodium hydroxide) because of its basic nature. But it still remains absent according to our bibliographic research the dosage of furosemide in plant extracts. The furosemide is a diuretic from the large family of alkaloids (fig.3), we extracted the total alkaloids from the plant according to the method used in 2010 by Houngbeme G.A in his Master of Research thesis. The extract obtained was contain the active ingredient. The figure below (fig. 4) summarizes the different steps of this extraction and the figure 5 shows liquid liquid fractionation with a separating funnel.



Furosemide

Figure 3:- Chemical structure of furosemide.

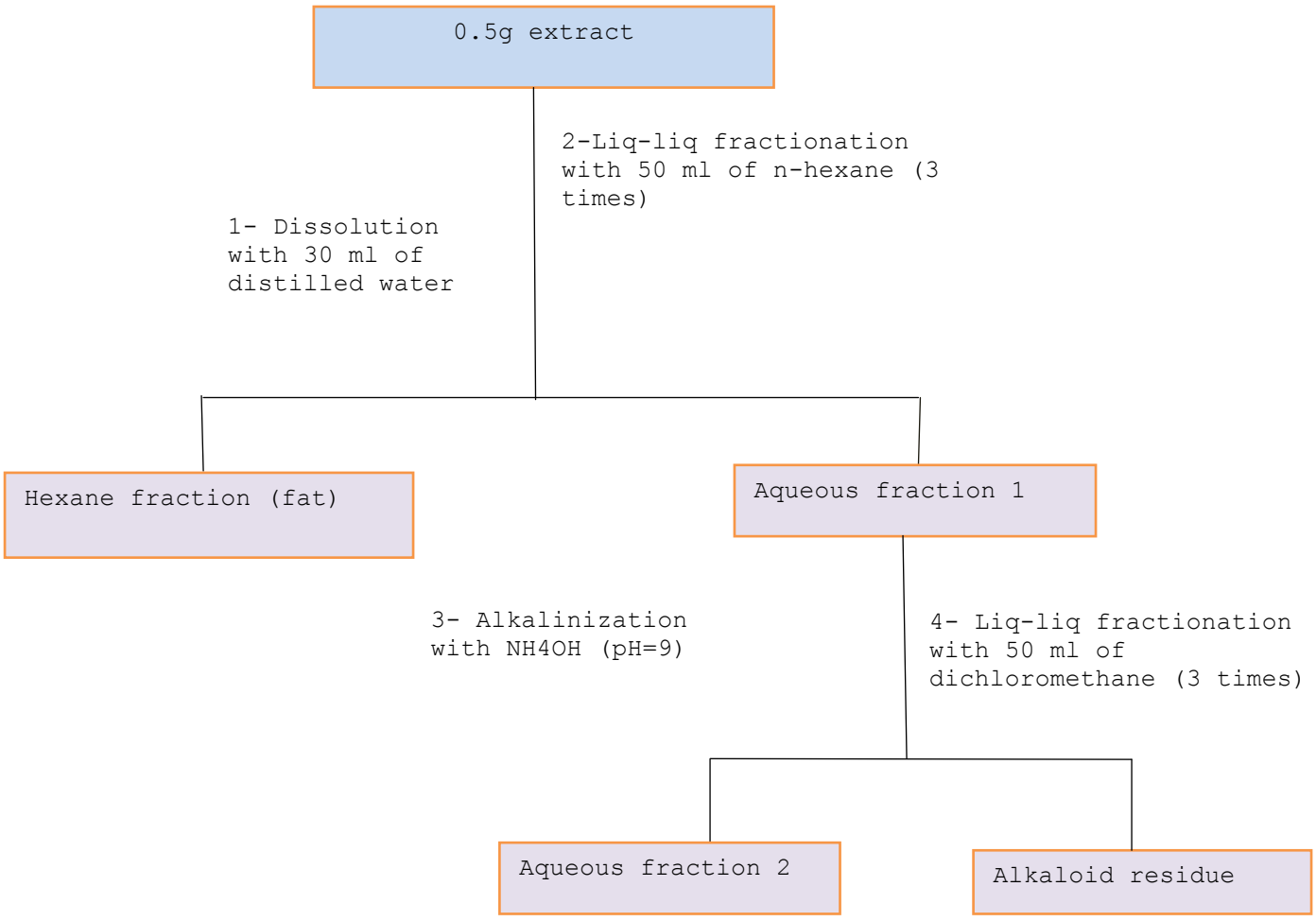


Figure 4:- Diagram extractionof furosemide.

Figure 5:- Separating funnel showing dichloromethane and aqueous fractions.

Identification and determination

The determination of furosemide in the extract were performed on a UV-Vis spectrophotometer with the spectrum model. Firstly,wecarried out the calibration from the 5 concentrations of dilute solutions of the active compound. The absorbance was read at 276 nm wavelength very close to that found 277 nm in the literature (Safila; 2014; Gahandule; 2016; Kalakuko; 2017). After calibration; the method was applied to the extract at a concentration of 12.5 µg/ml.

The different values of the device's response are reported in the equation of the regression curve in linear mode, to calculate the quantity of furosemide contained in the injected concentration. Subsequent calculations taking into account the test portion and the extraction volume made it possible to find the furosemide content of the analyzed extract.

Results:-

Yield of crude extract

After evaporation using a rotavapor and drying of the aqueous extract in an oven at 40°C; 12.57g of dry extract were obtained, i.e. a yield of 12.57%. We can say that some of the chemical principles of the plant have an affinity with water. There are therefore polar metabolites within the plant.

Analytical thin layer chromatography

After applying the analytical TLC (Fig. 6) in accordance with the method described above, the results show that these plant extracts contain several molecules materialized by spots visible (Figure 7), fluorescent under UV at 254 nm and at 365 nm (Figures 8 and 9).

Figure 6:- Development of TLC plate showing extract and standard deposits.

Figure 7:- TLC plate showing furosemide after mobile phase development.

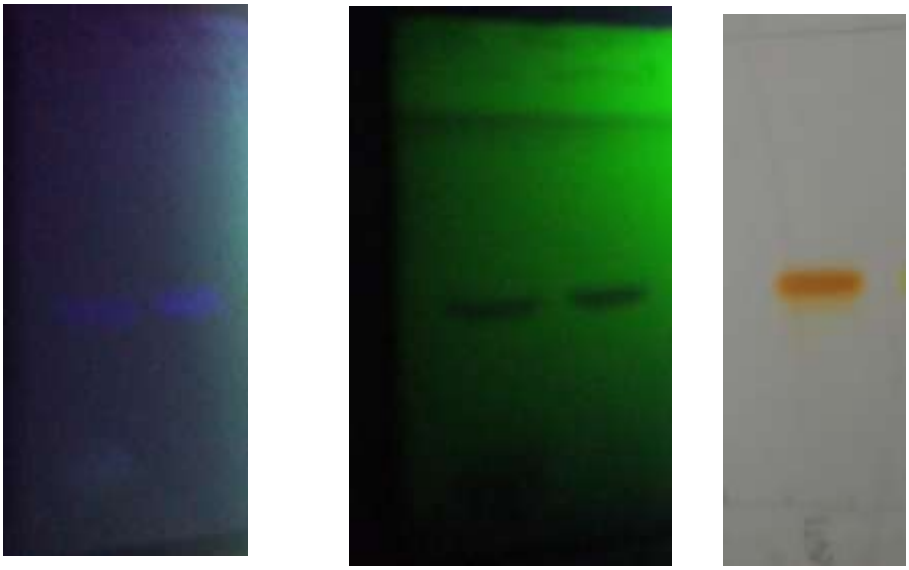


Figure 8:- TLC plate showing the molecules of the extract (left and middle) and furosemide (right) after development by the mobile phase (observation at UV 365 nm and 254 nm).

In order to justify that the mobile and stationary phases used allowed a good separation of the molecules on the plate, we calculated the frontal ratios (Rf) of the predominantly colored spots (Table 5).The frontal ratio is the quotient of the migration distance of a given spot compared to the distance from the front of the solvent which is 7cm (Houngbèmè et al.; 2015).

Table 1:- Rf value of furosemide visible spots.

spotlights	Xi	Rf= Xi/Xo
standard furosemide	3,21	0,45
furosemide in the extract	3,2	0,45

The aqueous extract analyzed shows a spot which appears at the same Rf=0.45 as that of standard furosemide which is an alkaloid. It is deduced that the root of C. roseus contains a priori furosemide.

Dosage of furosemide in the extract

Yield of liquid liquid fractionation

The different fractions obtained were evaporated; dried and weighed to calculate the yield, the values of which are summarized in Table 2 below:

Table 2:- Liquid Liquid Fractionation Yield.

fractions	Hexanic	alkaloid	aqueous
Yield **(%)**	**11,67%**	**4,58%**	**8 ,1%**

It should be noted that the extract is richer in fat. But the content of alkaloids in the extract is also greater when we know that alkaloids are often in very low quantities into the plants.

UV spectrum of furosemide

We did a 198-400nm scan (Figure 9) to see the level of absorption of the active ingredient.

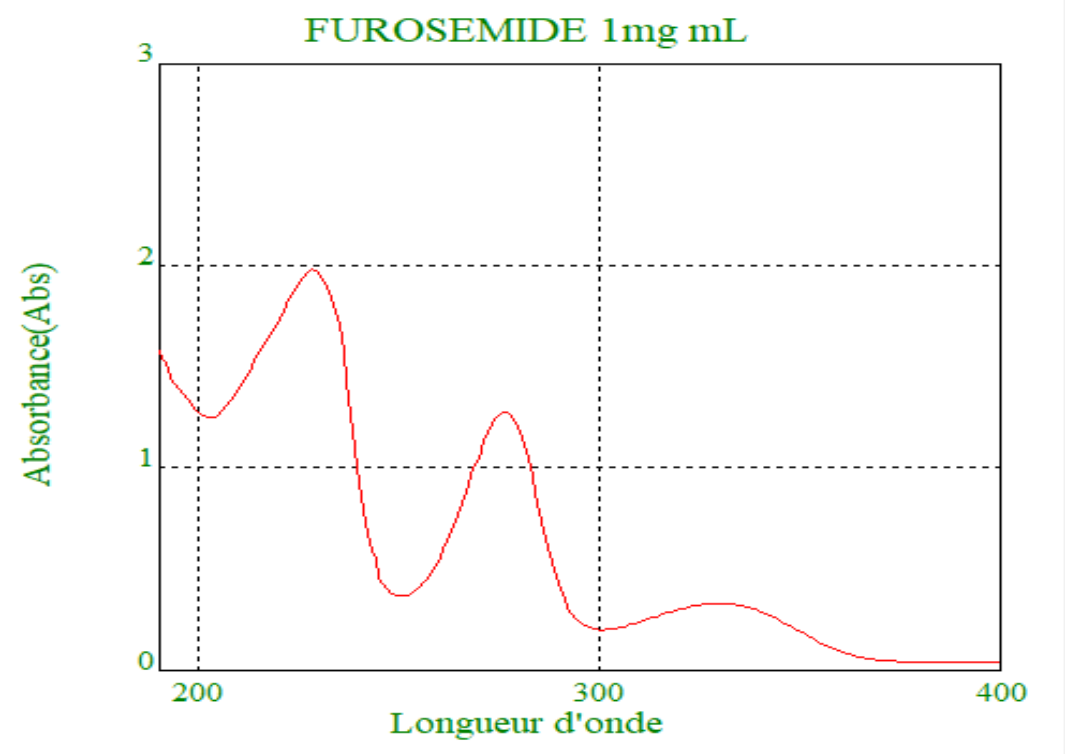


Figure 9:- Furosemide UV spectrum at 1000ppm.

The quantities injected and the mean of the optical densities made it possible to draw the calibration line (Fig.10).

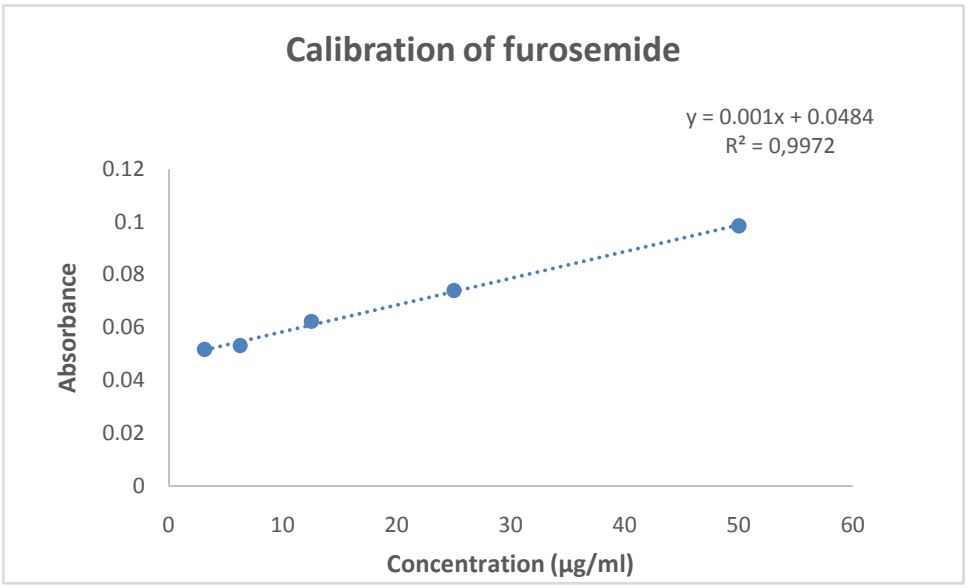


Figure 10:- Linear mode calibration curve of Furosemide.

The equation of this regression curve is Y= 0.001x + 0.0484 with a correlation coefficient r^2 = 0.9972. The correlation coefficient (r^2=0.9972) obtained shows that there is a good correlation between the optical density values obtained for each standard concentration injected. The calibration line equation can be used to calculate the amount of furosemide contained in the plant extract.
Y = optical density; x = (Y-0.0484)/0.001: concentration in µg/ml of furosemide.
The UV spectrum of the extract at 50ug/ml is shown in figure 10

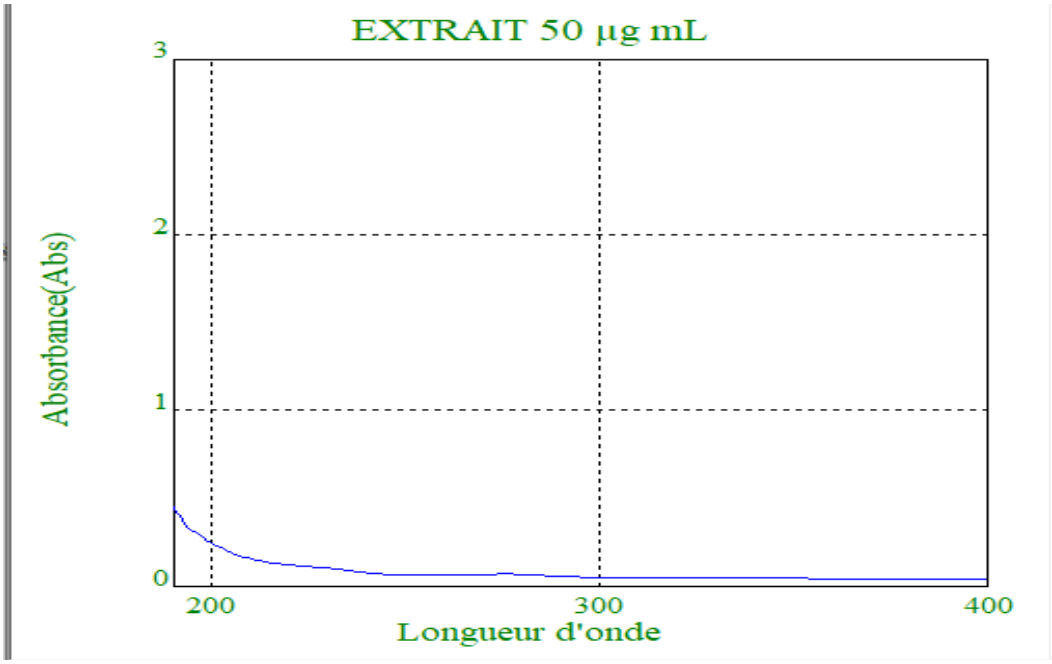


Figure 11:- UV spectrum of the extract at 276 nm.

Furosemide content
The overlaid spectra of the furosemide standard and the 276 nm extract are shown in Figure 12.

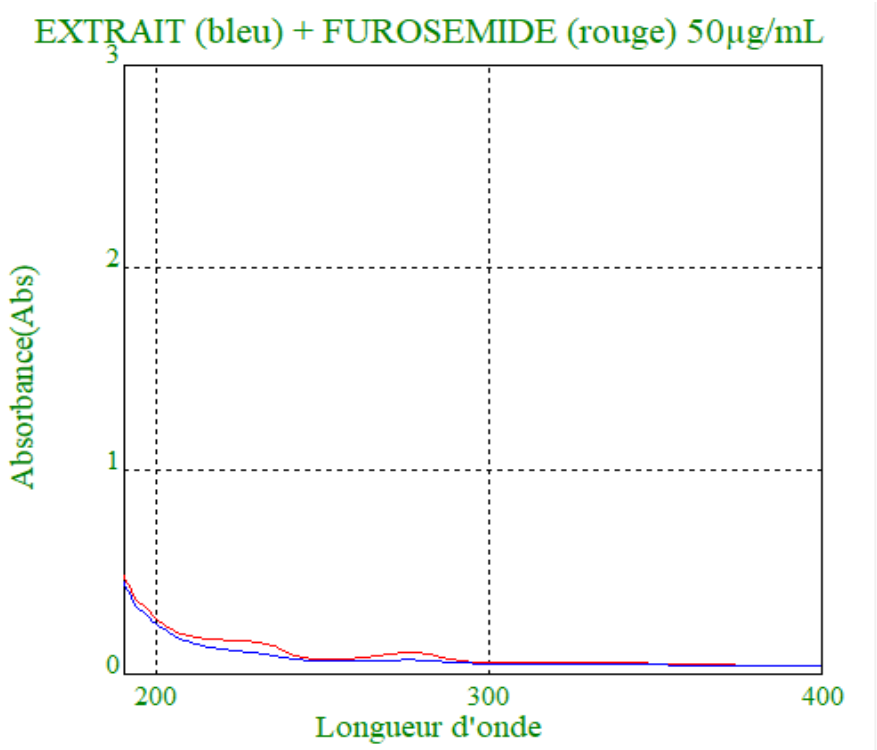


Figure 12:- Comparative spectra of furosemide and extract.

Figure 12 shows us that the standard-sample spectra recorded at 276 nm are well superimposed; and therefore, the aqueous extract of C. roseus contains the active ingredient furosemide.

The content in µg of furosemide per g of alkaloid extract calculated by taking into account the equation of the regression line; of the optical density read for the extract and the mass of the test sample is:

T= 1.27 ± 0.03 µg/g

Conclusion:-

The present study provides evidence that the roots of *Catharantus roseus* possess a diuretic property and therefore are used for the treatment of arterial hypertension. The relatively small amount of furosemide in the aqueous extract of the plant justifies to the presence of alkaloids in the roots of this plant. It is essential in the continuation of this work to evaluate in vivo the diuretic and antihypertensive activities of the extract in order to confirm again the use of this plant.

Aknowlogment:-

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Conflict of interest

The authors declare that there is no conflict of interest regarding this work.

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