Global Science Research Journals

ISSN: 2449-1802 Vol. 7 (3), pp. 280-283, July, 2019

http://www.globalscienceresearchjournals.org/

Global Journal of Medical, Physical and Health Education

Full Length Research paper

Development ways of detecting boxwood evergreens alkaloids by gc-ms method

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Accepted 24 July, 2019

Boxwood evergreen-Buxussempervirens L., widely used in ornamental horticulture. In homeopathy preparations prepared from leaves, boxwood is used as a diaphoretic, antifebrile and diuretic.The aim of the development ways for the analysis of cyclobuxin alkaloids, tug using the GC-MS method.We have used two methods to develop an optimal method for the extraction of cyclobouxin and buxinalkaloids from a plant sample. For the first method, raw material used and aerial part of the plant was dried. A sample of the raw material was ground to a particle size passing through a sieve with 1 mm diameter holes. The second method is a method for isolating alkaloids by infusion with oxalic acid solution.Two extracts were purified and for the separation of alkaloids by thin layer chromatography. For this, chromatographic plates, which are prepared in the laboratory, were utilized.On the chromatogram and mass spectrum, the main fragmentation ions characteristic of tow and cyclobuxin were identified.

Keywords: boxwood evergreen, cyclobuxinalkaloids, GC-MS method, buxinalkaloids, extract, chromatography, mass spectrum, ions of tow.

INTRODUCTION

Boxwood evergreen - Buxussempervirens L., widely used in ornamental horticulture. It is known that in homeopathy preparations prepared from leaves, boxwood is used as a diaphoretic, antifebrile and diuretic. It is also used in the treatment of rheumatism. However, it should be noted that all parts of the plant, especially the leaves, are very poisonous. The box contains about 70% of alkaloids, among the main ones you can mention tow, cyclobuxin, etc. The box contains the alkaloid buksin (found by Faure in 1830). In toxic amounts, buxin causes vomiting, diarrhea, clonic convulsions and death from respiratory failure. In the experiment, 750 g of boxwood leaves were fatal to the horse [1, 2].In the first 12-24 hours, death may occur due to the cessation of breathing. When autopsies of poisoned people are revealed, hyperemia and swelling of the mucous membrane of the stomach and intestines are usually observed [3].

Under natural grazing conditions, boxwood as a poisonous plant does not seem to matter much; unpleasant smell, taste and hard leathery leaves prevent eating animals from eating boxwood [4].

The naturalization of adventive species is a serious threat to the biodiversity of the colonized territory. According to the Russian authors [5], biological invasions of alien species can be attributed to environmental disasters. A. Demidova and G. Eremkin [6] note that as a result of a massive destruction, an evergreen box is a threatened disappearance - a tertiary relic, endemic to the Lazistan flora, listed in the Red Books of Russia, Georgia and Azerbaijan. According to the World Wide Fund for Conservation of Wildlife (WWF), by the end of 2016, out of 1528 local populations of boxwood on the southern macroslope of the Caucasus Mountains, only certain groups of plants survived in the valley of the Shah River [7].

Natural poisoning was observed in pigs, they arose as a result of eating branches of the boxwood, thrown out after cutting the bushes, or branches used as bedding.An analysis of the literature available to us has shown that to date no methods have been developed for isolating, detecting and determining box alkaloids in biological objects.

In this regard, we aimed tostudy the development of methods for the analysis of cyclobuxin and tow in biological objects by using the GC-MS method.

MATERIALS AND METHODS

We have used two methods to develop an optimal method for the extraction of cyclobouxin and buxinalkaloids from a plant sample. Below are these extraction techniques.

1-method. The raw material used was the dried aerial part of the plant. A sample of the raw material was ground to a particle size passing through a sieve with 1 mm diameter holes. About 10 g of crushed raw materials were placed in a flask with a capacity of 250 ml, 150 ml of diethyl ether and 7 ml of ammonia solution were added and the mixture was stirred for 1 hour. The ether extract was quickly filtered through cotton wool into a flask with a capacity of 200 ml, covering the funnel with a watch glass. 5 ml of water was added to the extract, it was vigorously stirred and left until the ether layer was clarified, after which the ether extract was transferred to a separator funnel with a capacity of 200 ml. From the ether extract, the alkaloids were re-extracted with a 1% solution of hydrochloric acid in portions of 20, 15, 10 ml each time, filtering each time through a paper filter moistened with water into another separating funnel of the same capacity. The filter was washed twice with a 1% solution of hydrochloric acid in 5 ml portions, attaching the washings to the total extract. Acidic re-extract was made alkaline with concentrated ammonia solution until alkaline (pH=9) using phenolphthalein and the alkaloids were extracted with chloroform successively in portions of 20, 15, 10 ml. The chloroform extracts were filtered through a filter paper containing 4-5 g of anhydrous sodium sulfate. Chloroform was distilled off at room temperature. The dry residue was dissolved in 1 ml of ethanol. This solution was used for further research.

2-method. The literature describes a method for isolating alkaloids by infusion with oxalic acid solution. The raw material used was the dried aerial part of the plant. Crushed to particle size, passing through a sieve with holes with a diameter of 1 mm. About 10 g of crushed raw materials were placed in a flask with a capacity of 250 ml, 150 ml of 2% solution of oxalic acid were poured in, repeated precipitation of 7 ml of ammonia solution and the mixture was shaken for 1 hour. The oxalic acid extract was quickly filtered through cotton wool into a flask with a capacity of 200 ml. Alcohol was added to the extract, followed by a saturated sodium chloride solution. Basified with 25% ammonia solution to pH = 9. From this mixture, the alkaloids were extracted with diethyl ether in succession in portions of 20, 15, 10 ml, shaking for 3 minutes. The ether layer was separated, each time filtering through a filter paper moistened with diethyl ether. The ether was distilled off at room temperature. The dry residue was dissolved in 1 ml of ethanol.

The resulting two extracts were purified and, accordingly, for the separation of alkaloids by thin layer chromatography. For this, chromatographic plates prepared in the laboratory were used.

As the mobile phase was used a mixture: ethyl alcohol: diethyl ether, (8: 2). For the development of the zone of localization of alkaloids used several reagents. The best developer for tow and ciclobuxin were the Dragendorff modified according to Mounier (orange-red spot), the azo coupling reaction (when applying sodium nitrite solution, 10% hydrochloric acid and an alkaline solution of β-naphthol, red staining was determined). Also, when viewed under UV rays of 254 nm, dark brown spots were observed. After we had developed, the Rf value of our test solution was 0.72 in the alcohol extract and Rf=0.48 in the ether extract.

From the alkaloids localization zones, each alkaloid was eluted separately with a solvent mixture of chloroform-methanol (95: 5). Each eluate was dried to dryness at room temperature. The dry residue was dissolved in 5 ml of ethanol and analyzed by gas chromatography / mass spectrometry. The samples were analyzed on an Agilent Technology GC 6890 chromato-mass spectrometer with a 5973N single-quadrupole mass-selective detector using a capillary column measuring 30 mx 0.25 mm x 0.5 μm with 5% phenylmethylsiloxane in dimethylsiloxane at the following analysis conditions: injector temperature - 280 ° C , the MS source temperature is 230 ° C, the MS of the quadrupole is 180 ° C, when programming the column thermostat temperature: the initial 80 ° C exposure time is 2 min, then to 270 ° C with a temperature rise rate of 10 ° C / min, helium carrier gas, flow rate 1 ml / min magnitude in 5 .mul sample, sample input mode splitless. Peak identification was performed by comparing the mass spectra of the peaks with the NIST11.L, W10N11_Full.L mass spectra libraries available in the database, Wiley275.L.

RESULTS

On the chromatogram and mass spectrum, the main fragmentation ions characteristic of tow and cyclobuxin were identified: 18.56; 19.76; 20.18; 24.10; 24.58; 26.31; 29.17 m / z (figure 1).

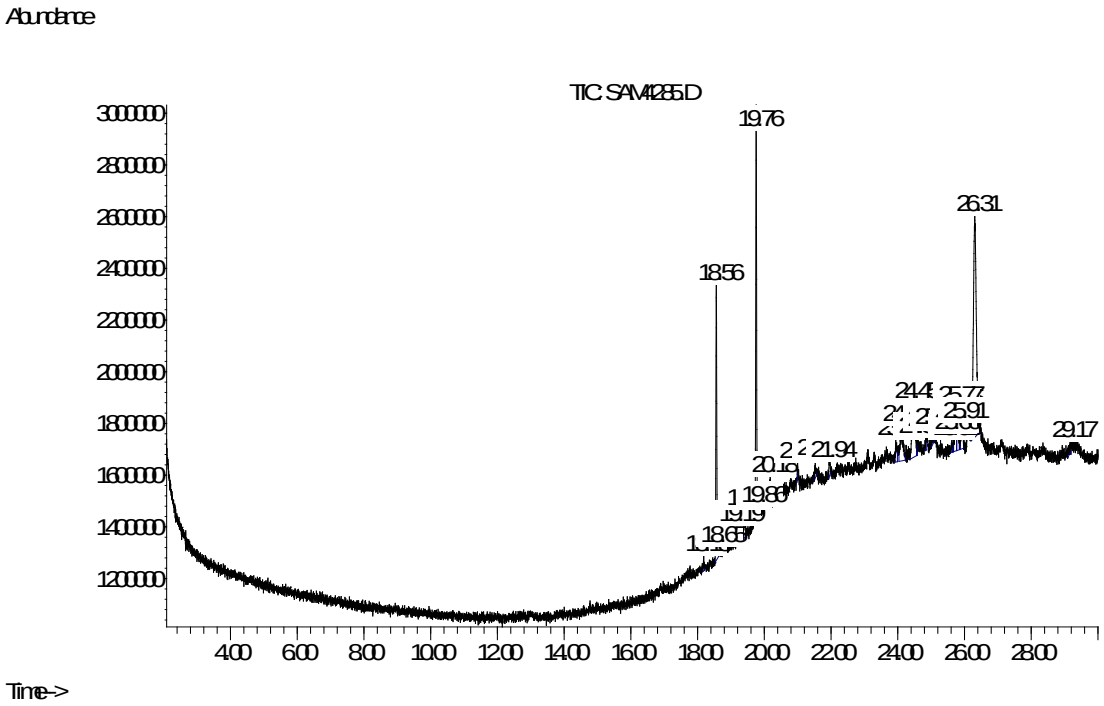

Figure 1: Chromatogram of chloroform extract boxwood evergreen

To establish the suitability of the method in the chemical-toxicological analysis of alkaloids, model samples of bioliquids (blood and urine), etched with boxwood extract, were prepared. Alkaloids from bioliquids were extracted with chloroform (1-method) and ether (2-method). Under the above conditions, the chromatographic method was used and the resulting eluates were analyzed by GC-MS. As a result, it was established that chromatographic peaks and mass spectra of alkaloids of biological liquids isolated from plant materials and model objects coincided in retention time and m / z values of fragment ions (Figure 2 and 3).

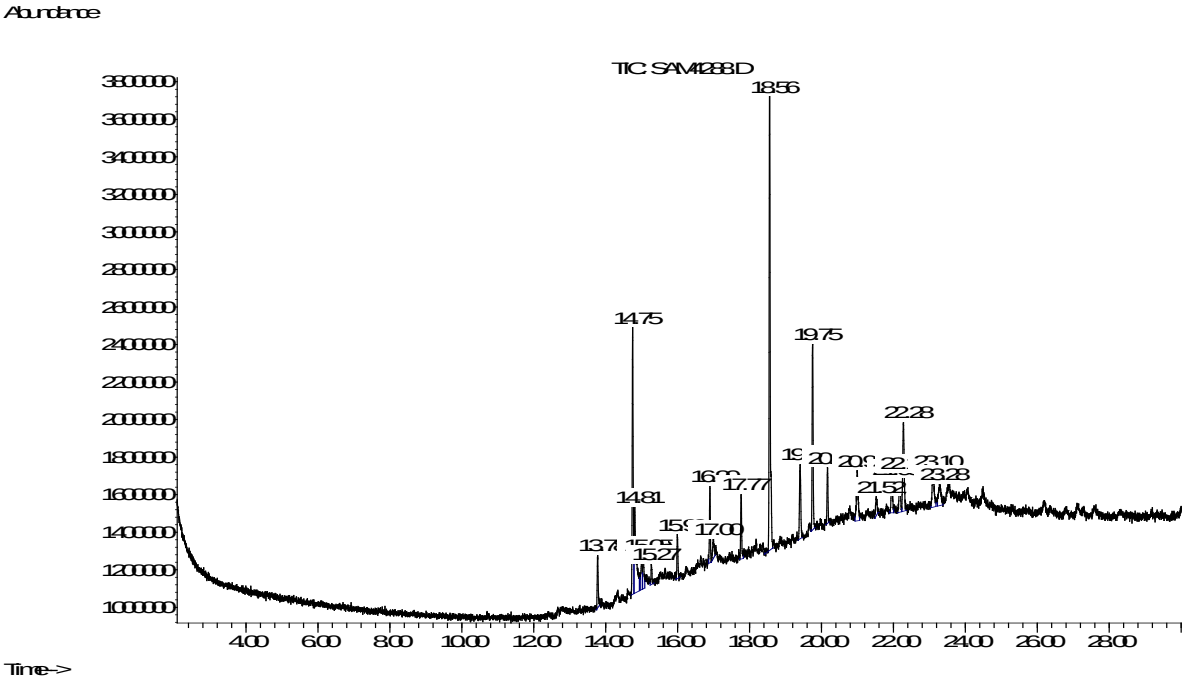

Figure 2: Chromatogram of chloroform blood extract etched with boxwood extract

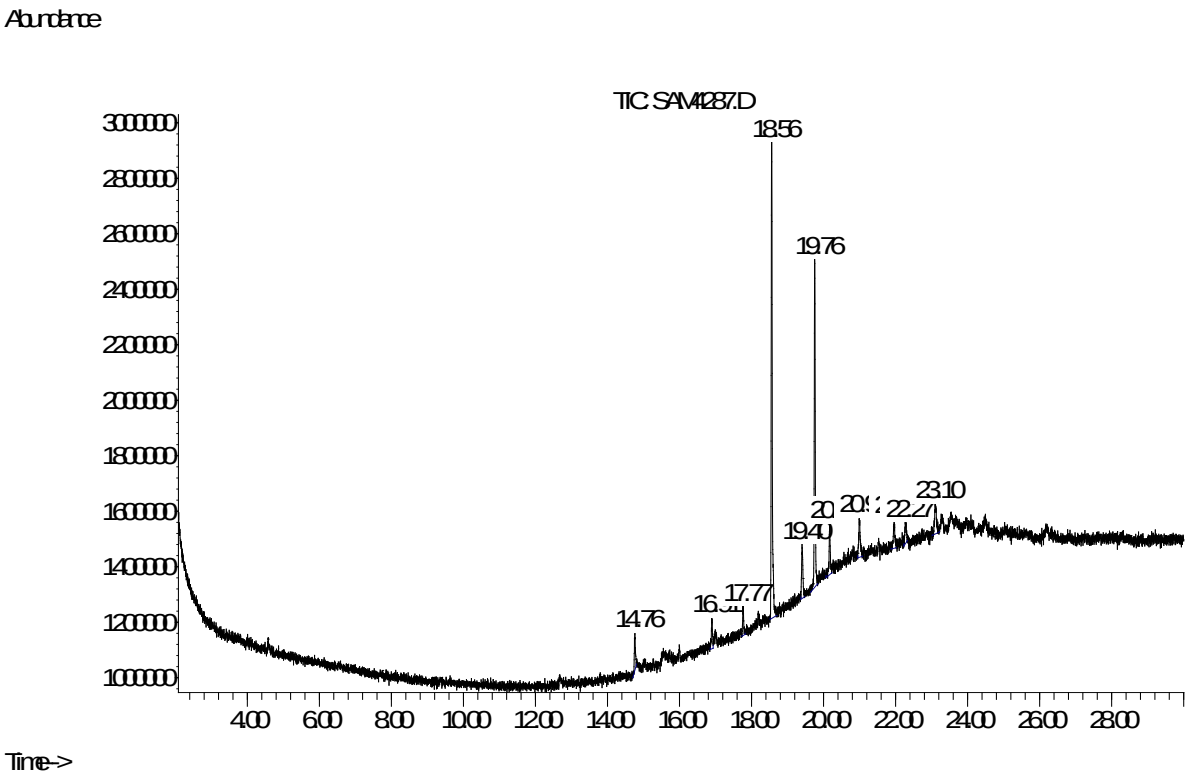

Figure 3: Chromatogram of urine chloroform extract pickled boxwood extract

CONCLUSION

Evergreen box alkaloids — buxin and cyclobouxin were extracted using two isolation methods. The conditions for detection by the GC-MS method of buxin and cyclobuxin isolated from vegetable raw materials have been developed. The developed conditions of the GC-MS method were used to detect the alkaloids in model liquids etched with the evergreen boxwood extract.

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