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Quality control and anticholinesterase activity determinations on Sternbergiasicula

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ABSTRACT: Two distinct vegetation phases, the blooming and fruiting seasons, were used to harvest aerial and subsurface portions of SternbergiasiculaTineo ex Guss. fromSöke (Aydın). The specimens that were created were subjected to quality control investigations and anticholinesterase activity tests. Drug specimens made independently from plants taken throughout blooming and fruiting phases were subjected to tests for humidity, total ash, hydrochloric acid-insoluble ash, and sulphated ash as part of the quality control investigations.Samples varied in humidity (7.828–8.798%), total ash (7.086–16.024%), hydrochloric acid–insoluble ash (1.120–4.340%), and sulphated ash (11.022–23.465%). A titrimetric approach was used to determine the total alkaloid contents of specimens of Sternbergiasicula. The total quantity of alkaloids varied from 0.122 to 0.496 percent. The herba of S. sicula that was harvested during the blooming time had the greatest concentration of total alkaloids. The herba of had the lowest total alkaloids concentration.The fruiting season is the best time to gather S. sicula. In addition, the entire alkaloid extracts from the drug specimens were tested for anticholinesterase activity using a Thin Layer Chromatography (TLC) assay, which is based on the in vitro Ellman technique. The anticholinesterase action was shown by all of the alkaloidal extracts.

KEYWORDS:*Sternbergiasicula*,Amaryllidaceae,QuantitativeDetermination, Anticholinesterase Activity

INTRODUCTION

There are six different species of SternbergiaWaldst& Kit., often known as winter daffodils, in Turkey (1,2). Members of this genus, including S. sicula, may be found all throughout the Mediterranean, including Greece, Sicily, the Aegean, and the eastern Mediterranean (3). Compounds from the skeletally distinct families of Amaryllidaceae alkaloids were isolated from S. sicula in earlier investigations (4–7). The alkaloids found in the amaryllidaceae family have many useful biological functions, such as inhibiting acetylcholinesterase, fighting viruses, and even tumors (8-12). Sternbergia species have been the subject of numerous studies on their phytochemistry and bioactivity (13–16), but no studies have documented quality control determinations for these species. This information could provide a foundation for future monographs on Herba and Bulbus drugs made from these plants.

We conducted many quality control tests to determine the standards for medications synthesized from the aerial and subsurface sections of Sternbergiasicula gathered during two separate vegetative stages as part of our continuing study on Sternbergia species of Turkish provenance. For the gravimetric measurements of humidity, total ash, hydrochloric acid-insoluble ash, and sulfured ash, as well as for the titrimetric measurements of total alkaloidal content, the European Pharmacopoeia was consulted (17).

The acetylcholinesterase inhibitory action of plants and alkaloids from the Amaryllidaceae family has been the subject of much research (10, 11, 18). One alkaloids from this family, galanthamine, is used to treat Alzheimer's disease (AD) because of its anticholinesterase activity.

that year (19). The cholinergic hypothesis states that AD is associated with a cholinergic system deficiency (18). Thus, blocking acetylcholinesterase (AchE) is a crucial strategy for treating Alzheimer's disease (AD). Consequently, looking for

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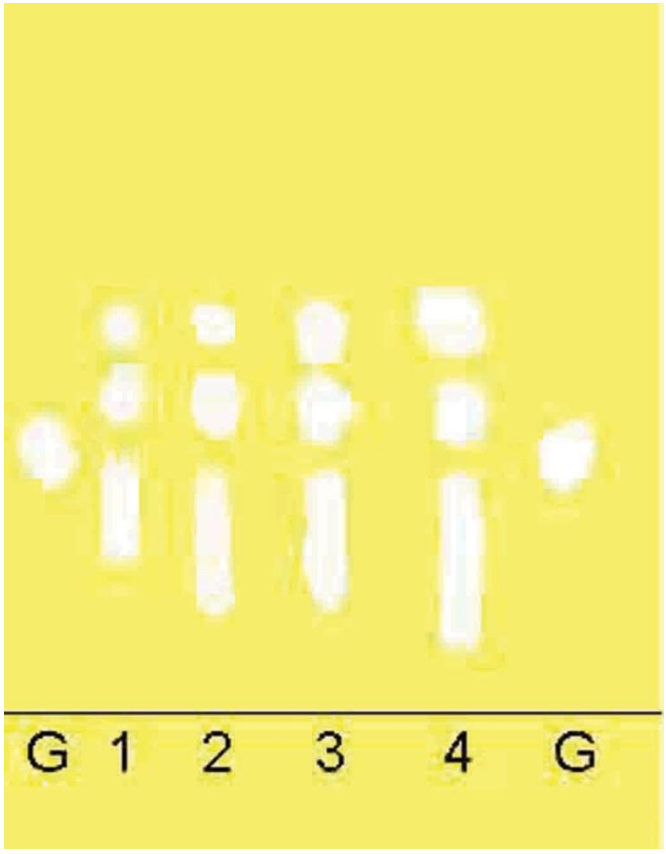

FIGURE1: Acetylcholinesteraseinhibitoryactivityofalkaloidalextractsof*Stern- bergiasicula*.G:Galanthamine,1:Bulbus/flowering,2:Herba/flowering,3:Herba/ fruiting, 4: Bulbus/fruiting

AchEinhibitorsfromplantsincludingAmaryllidaceaespecies hasgainedimportanceinthelastdecade.Inthiscontext,alka- loidal extracts prepared from the aerial and underground parts of *Sternbergiasicula*, collected at two different vegetationperiodswere screened for theirAchE inhibitory activity byus-ing a thin layer chromatography (TLC) assay based on Ell- man's method (20) which is a quite simple and also effective method to identify active extracts and/or compounds.

MATERIALSANDMETHODS

PlantMaterial

*S. sicula*was collected from Söke (Aydın) during flowering and fruiting seasons in November 2007 and March 2008, re-spectively. The plant was identified by Prof. M. Ali Önür from theDepartmentofPharmacognosy,FacultyofPharmacy,Ege University, Izmir (Turkey). Voucher samples of *S. sicula*(No. 1388, 1389) are deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

Aerial and underground parts collected during two different vegetationperiods,wereseparated,cutintomoderatelysmall pieces and dried in shadow at room temperature.

Humidity, Total Ash, Hydrochloric Acid-Insoluble Ash, SulphatedAshandTotalAlkaloidContentDeterminations

EuropeanPharmacopeiawasreferredtoforthegravimetricassaysofhumidity,totalash,hydrochloricacid-insolubleashandsulphatedash.Thetotalalkaloidcontentof eachspecimen wasevaluatedbyusingatitrimetricmethodcitedinEuropean Pharmacopeia for various alkaloid-containing drugs (17).

AlkaloidExtraction

6 g of accurately weighed powdered plant material was mac-eratedwith100mLEtOHfor24hours,andthenextractedfur-therwithEtOHuntilnopositivereactionisobservedwiththe DragendorffandMayerreagents(17)**.**Afterevaporationofthe solvent, the residue was dissolved in 50 ml portions of 1 % aqueoushydrochloricacid(250mLintotal)andfiltered.Com-binedacidicfiltrateswerewashedwith3x100mL petroleum ether (40-60˚), made alkaline with 26 % ammonium hydroxide (pH 9-10) and extracted with 6x100 mL chloroform until the organic solvent displayed no positive reaction with Dragen-dorffandMayerreagents.Thecombinedchloroformextracts were then dried over anhydrous Na_2SO_4, filtered, and the or-ganicsolventdistilledinvacuotoaffordthealkaloidalextract. 0.02 N H_2SO_4 solution was added to this extract and kept onwater bath (50-60˚C) until the extract was completely dis-solved. Then three drops of methyl red reagent (17) was addedand the solution was titrated with 0.02 N NaOH. The proce-dure was carried out in a series of three parallel experiments.The mean results are given in Table 1.

Chemicals

Acetylthiocholine iodide (ATCI), Acetylcholinesterase enzyme(AChE) Type VI-S: From Electric Eel, 5,5-dithiobis [2-nitroben-zoic acid] (DTNB) were obtained from Sigma. Tris-HCl waspurchased from Merck. Galanthamine was isolated from sev-eralAmaryllidaceae species in our laboratory and authenti-catedbymeansofspectralanalysis(UV,IR,MS,NMR)(21).The other reagents were of analytical grade.

AcetylcholinesteraseInhibitoryActivityDeterminations

TLC assay combined with bioactivity staining for AChEinhi-

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bition was modified from a previous study (18). A 2.5 mm Silica gel plate F_{254}(0.2 mm, Aluminium sheet, Merck) was usedas a stationary phase. The plant extract (10 mg/ml) and galan-thamine (1.5 mg/ml) dissolved in chloroform-methanol (8:2),were spotted on the TLC plate and it is developed in the mo-bile phase benzene-chloroform-methanol-ammonium hydrox-ide (26 %) 8:9:3:2 drops (v/v/v/v). After the plate was dried atroom temperature, it was sprayed with 1mM ATCI and1mMDTNBinTris-HCl,pH:8,andupon3-5minutesdrying,theplatewassprayedwith3Unit/mlAChEinTris-HCl,pH:8.After20minutesayellowbackgroundappeared;occurrenceof white spots indicated positive reaction.

RESULTSANDDISCUSSION

Inthecourseofthestudiesonqualitycontrol,humidity,total ash, hydrochloric acid- insoluble ash and sulphated ash were determined for drug specimens prepared separately from plants in flowering and fruiting periods.

Theresultsofthehumidity,totalash,hydrochloricacid-insoluble ash and sulphated ash assays suggested that it was appropriate to include these criteria in a prospective monograph onHerbaandBulbusdrugsthatwouldbepreparedfromthis plant, and the present findings might be utilized in the establishment of standard values during the elaboration of these monographs (Table 1).

TABLE1. ResultsoftheQuality ControlDeterminationsCarriedon*Sternbergiasicula*

Specimen*	Humidity(%)§	TotalAsh (%)§	HydrochloricAcid-InsolubleAsh(%)§	SulphatedAsh(%)§	TotalAlkaloids(%)§
1	8.480 ±0.088	14.054 ±0.201	3.113 ±0.092	23.465 ±0.263	0.308 ±0.011
2	7.828 ±0.084	9.280 ±0.057	1.120 ±0.103	15.117 ±0.120	0.496 ±0.012
3	8.798 ±0,067	16.924 ±0.074	4.340 ±0.237	21.225 ±0.211	0.122 ±0.011
4	8.742 ±0,156	7.086 ±0.038	2.659 ±0.091	11.102 ±0.069	0.236 ±0.007

*1:Bulbus/flowering;2:Herba/flowering;3:Herba/fruiting;4:Bulbus/fruiting
§MeanResults±StandartDeviations

An indicator of Sternbergiasicula quality might be its total alkaloidal content. Herba of Sternbergiasicula obtained during the fruiting season had the lowest levels of alkaloids, whereas herba collected during the flowering season had the highest values, ranging from 0.122 to 0.496% (Table 1). Quantification of total alkaloids in Sternbergia species has been documented in a small number of papers (22). Unfortunately, the total alkaloids in Stern-bergiasicula have not been quantified in any published findings. Previous reports have detailed the total bases and galanthamine content of some Sternbergia species as well as the results of isolation investigations.24 and 23. The most quantifiable alkaloid in Sternbergia species is lycorine, according to a comprehensive literature search (25, 26). The concentration of lycorine in many Amaryllidaceae species, including S. sicula, has been recently quantitatively measured by HPLC-DAD analysis (27).

The in vitro Ellman technique was used to test the total alkaloidal extracts obtained from drug specimens for anticholinesterase activity, in addition to quality control and total alkaloid determinations. Spraying with DTNB/ATCI reagent first made the white spots visible, and then spraying with AchE reagent, which produced a yellow background, confirmed the existence of anticholinesterase activity. Active extracts and recognized chemicals may be determined qualitatively using this technique. Figure 1 shows that all of the S. siculaalkaloidal extracts exhibited anticholinesterase activity.

We have not previously detected galanthamine in S. sicula in our HPLC investigations of this plant (28). Thus, the current study's findings suggest that, in addition to galanthamine, alkaloids with anticholinesterase action were found in the alkaloid extracts made from S. sicula bulbs and aerial parts collected at various stages of growth. This conclusion is further supported by the well-documented anticholinesterase activity of many alkaloids discovered in Sternbergia species (4-7) in the literature (18, 29).

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