Journal of Animal and Veterinary Advances 13 (15): 905-907, 2014
ISSN: 1680-5593

Extraction of Alkaloids from *C. komarovii* Al. Iljinski

[1, 2]Alaa. H. Sadoon, [1]Xiwang Liu and [1]Jiyu Zhang
[1]Key Laboratory of Veterinary Pharmaceutical Development,
Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS,
Ministry of Agriculture, 730050 Lanzhou, P.R. China
[2]College of Veterinary Medicine, University of Basrah, 61001 Basrah, Iraq

Abstract: *Cynanchum komarovii* Al. Iljinski is a plant belonging to Asclepiadaceae family, distributes widely in the grassland of Northern China and has been used for preventing and reducing inflammation as a traditional Chinese medicine. The phytochemical screening for *C. komarovii* plants revealed a number of bioactive constituents including alkaloids, flavonoids, polysaccharide, terpenes, fatty acid, phospholipids, amino acids and vitamin. Researchers have been identified thirteen types of alkaloids from *C. komarovii*. Therefore, the present study aimed for investigating quantity of alkaloids in these plants and best methods of extracting crude alkaloids from these plants. Three methods was used for extracting alkaloids from *C. komarovii* plants, the first methods depended on soaking the plants in 95% alcohol and water, evaporating out the alcohol and adding HCL. The second method depend on soaking the plants in 95% alcohol without water and the third method was depend on soaking the plants in 3 times 95% industrial alcohol and 1 L water that contain 10 mL of HCl and leaves at room temperatures for 12 h. Results of extraction of alkaloids from *C. komarovii* plants revealed the best methods for extraction was the third methods and extracting yield by this was 2.3 g alkaloids for each 1 kg of plants and this represent high quantity that researchers reach for it in the study. Extraction total alkaloid from *C. komarovii* becomes more perfect by mixing acidic solvent with water and alcohol.

Key words: HCL, *C. komarovii*, Asclepiadaceae, vitamin, China

INTRODUCTION

Plant-derived substances have recently become great interesting owing to their versatile applications. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al*., 2008). Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products obtained from plants are relatively complex mixtures of metabolites in liquid or semisolid state or (after removing the solvent) in dry powder form and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician (Remington, 2006). The phytochemical screening for *C. komarovii* plants revealed a number of bioactive constituents including alkaloids, flavonoids, polysaccharide, terpenes, fatty acid, phospholipids, amino acids and vitamin (Sun *et al*., 2012). The term of alkaloids can apply to the naturally occurring organic containing one or more heterocyclic nitrogen atoms in the molecule. This concept was first proposed by a pharmacist, W. Meissner in 1819 to cover a group of naturally occurring substances found in plants. Alkaloids are often extracted by acid-base shake out technique (Oguegbulu and Uche, 2012). However, the course of which is influenced by a number of factors. Hydrochloric acid, combined with industrial alcohol is a nontoxic, non-inflammable solvent system and it is generally considered to be environment-friendly (Li and Wu, 2007). The aim of the present study focused on perfect methods for extraction more quantity of alkaloids from *C. komarovii* plant by acid-base shake out technique.

MATERIALS AND METHODS

Plant material: The study was carried out in Lanzhou Institutes of animal science and veterinary pharmaceutics,

Corresponding Author: Jiyu Zhang, Key Laboratory of Veterinary Pharmaceutical Development, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Ministry of Agriculture, 730050 Lanzhou, P.R. China

Chinese Academy of Agricultural Science, China from September 2012 to November 2012. The aerial parts and roots of *C. komarovii* were collected between July and August 2012 from Inner Mongolia, China and the whole plants was identified by Professor Da Nengatai from Animal toxicopathology and cure institution of Alashan county Inner Mongolia. *C. komarovii* were cleaned and dried under shaded condition for a few days. Following drying they were crushed into a fine powder which was then kept in container.

Solvents: Choice of solvents and successful determination of biologically active compounds from plant material largely depended on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes many factors such as low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractant (Parekh *et al*., 2006). The most solvents used in the experiments were water, Hydrochloric acid (HCl) industrial ethyl alcohol 95%, Sodium Hydroxide (NaOH) and chloroform.

Method of extraction: Powder of aerial parts and roots of *C. komarovii* were soaked three times with 95% industrial alcohol and then added 1 L water that contain 10 mL of HCl and leaves at room temperatures for 12 h then ultrasonicated for 60 min at 44°C. The combined matter was filtrated and concentrated by evaporator for separating alcohol from alkaloids and other components of plants. The extraction containing different component was mixed with water (3:8) and filtrated it by Whatman filter study No. 1 to remove fat soluble substances and other components.

The solution was then modified to pH 9-11 by Sodium Hydroxide (NaOH) and then liberated the alkaloids by extracted it with chloroform 3-5 times and then evaporated the chloroform to liberated chloroform extract. The chloroform extract was dried with anhydrous Na_2SO_4 and evaporated under vacuum to yield crude alkaloid extract according to (Fig. 1).

Method of detecting alkaloid in extracting materials: For detection that this materials that getting from extraction are alkaloids researchers used Dragendorff's reagent that

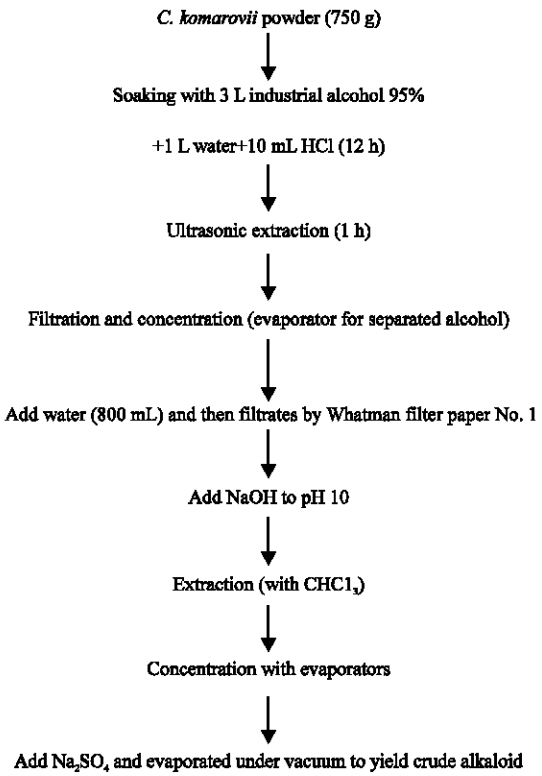

Fig. 1: Alkaloids extraction stages from *C. komarovii*

purchased for Sigma-Aldrich as in fellow. For each 2 mL of extracted chloroform, 5 mL of distilled water was add, 2 mL hydrochloric acid was add until acid reaction occurs then added 1 mL of Dragendorff's reagent for this mixtures. Formation of orange red precipitate indicates the presence of alkaloids (Kodangala *et al*., 2010).

RESULTS AND DISCUSSION

The alkaloids in plant material naturally exhibit a variety of physics, biological as well as medicinal properties. In plant materials, alkaloids generally occur partially as free bases and partially as salts which are insoluble in most of the organic solvents. The basic compounds form their crystalline salts with acids like hydrochloric acid, sulphuric acid and tartaric acid. The free alkaloids are insoluble or slightly soluble in water but their salts are freely soluble. However, they are soluble in less polar solvents such as hexane, ether or chloroform. (Rahman *et al*., 2011).

Three methods was used for extracting alkaloids from *C. komarovii* plants, the first methods depended on soaking the plants in 95% alcohol and water, evaporating out the alcohol and adding HCL. The second method depend on soaking the plants in 95% alcohol without water and the third method was mentioned in methods.

The results revealed the best quality and more quantity of alkaloids in third methods due to hydrochloric acid with water and alcohol permits for alkaloids to separating from plants and forming salts and precipitate at the bottom of containers, repeating soaking the plants 3 times permits for all alkaloids for extracting from plants.

This result was agree with Congai *et al.* (2013) that demonstrated when acid and water were the extraction solvents, the extraction rate to alkaloids was relatively high but there were problems with mildew and forming changes of the raw material during soaking and extracting. So that it will effect on extracting materials and the process of filtration through Whatman filter study become more difficult.

Researchers solved this problem during the experiment by determine the amount of alcoholic extraction which is acidic in natures and facilitate the process of filtrations by add more water as (3:8) because this will decrease pH degree and making the solubility of contents more easily this theory was agree with Verma *et al.* (2007) that mentioned through extracting alkaloids from *Catharanthus roseus*, alkaloids form salts in aqueous acidic media which improved solubility and enhanced stability at low pH value in addition, protons in the aqueous acidic media assist in breaking the sample matrix to release the analytes more easily.

The present study was demonstrated that the total extracting yield of alkaloids from *C. komarovii* in present study about 95 g from 40 kg of whole plants that equail to 2.3 g from each 1 kg plants. This quantity of extracting alkaloids is higher than (Guo *et al*., 2014) mentioned each 1 kg of *C. komarovii* plants giving 2 g of extracting alkaloids and the reasons may be related to using just ethanol 75% for soaking the plants materials.

The results showed these extracted alkaloids can react with Dragendorff's reagent and formed orange red precipitate, moreover these alkaloids also experimentally don't dissolve in water and just dissolve in alcohol.

CONCLUSION

Extraction total alkaloid from *C. komarovii* becomes more perfect by mixing acidic solvent with water and alcohol. The amount of extracting alkaloids was 2.3 g from each 1 kg plants, therefore the extracting methods must continue for investigating perfect methods for extraction more alkaloids from this plant.

ACKNOWLEDGEMENTS

Researchers appreciate the kind support of Animal toxicopathology and cure institution of Alashan county Inner Mongolia. The project was supported by the earmarked fund for China Agriculture Research System (CARS-38).

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