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Annals of Chromatography and Separation Techniques

Research Article

Isolation and Identification of Alkaloids extracted from Local Plants in Malaysia

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Abstract

The purpose of the study was to isolate and identified the alkaloids fractions local plants in Malaysia. TLC of alkaloid extracts from the plants used in this study revealed the presence of these compounds by using Dragendroff's reagent to reveal characteristic orange bands of alkaloids. IR spectra of alkaloids extract from the fruit of *M. citrifolia*, leaves of *A. squamosa*, and the roots of *A. Angustiloba* exhibited a strong O-H from the fruit of *M. citrifolia*. C-H stretching groups are shown for the fruit of *M. citrifolia*, the leaves of *A. squamosa* as well as the roots *A. angustiloba*. The N-H groups are showed in the fruit of *M. citrifolia* and the root *A. angustiloba*. The C=O bond at the leaves of *A. squamosa* and the roots of *A. angustiloba*. C-H group bonds were detected for the fruit of *M. citrifolia*, the leaves of *A. squamosa* and the roots of *A. angustiloba*. It could be concluded that the alkaloids of the plants can be a new source of antimicrobials against pathogenic bacteria and antioxidant source.

Article Information

Received date: Feb 26, 2016
Accepted date: Mar 15, 2016
Published date: Mar 16, 2016

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Keywords Local plants; Alkaloids; TLC; IR

Introduction

Morinda citrifolia is one important plant used as a medicine in many countries of the world. The common names of this plant are Noni, Indian mulberry, *nuna*, and *mengkudu* [1,2]. A medium-sized tree, it is 3-10 metres tall with abundant wide elliptical leaves and small tubular white flowers, which are grouped together. The petioles leave ring-like marks on the stalks [3,4]. The oval-shaped fruit of this plant has an embossed appearance. It is initially green to yellow in colour but the ripe fruit is white and covered with small reddish brown buds containing seeds [5]. The seeds are medium sized, ovoid in shape, reddish brown and with a distinct air chamber at the end probably for widespread seed dispersal by water [1,2,6]. This plant is found in South East Asia, Caribbean countries, Australia and Central-South America [1,3]. *M. citrifolia* has been used as a medicine for many ailments such as dysentery, heartburn, liver diseases, diabetes, high blood pressure, muscle aches, headaches, heart diseases, cancer, gastric ulcers and arthritis. It has also been applied for the treatment of drug addiction. The ripe fruit of this plant is used to treat respiratory infections and tuberculosis [7]. The roots and bark of *M. citrifolia* can be turned into dyes and medicine, while the leaves and fruit are sources of food and medicine [1,2]. The juice from the fruit of this plant has a long medicinal history in places such as the Fiji Islands, the Pacific Islands, South-east Asia and India [8]. Through *in vivo* experiments, Glang, et al. [9] reported that noni juice was deemed to be effective in the treatment of gingivitis and periodontitis. It used twice daily as a mouthwash, this juice significantly reduced the gingival inflammation.

The second plant is *A. squamosa* which belongs to the Annonaceae family and its common names are Nona, sugar apple, ata, gishta and sweet sop plant [10,11]. The genus *Annona* comprises 120 species. An economically significant species is *A. squamosa* which belongs to the Annonaceae family. Its specific native range is indefinite because of widespread commercial cultivation but is generally deemed to originate from the Caribbean region [12]. Common names for this plant are Nona, sugar apple, ata, gishta and sweet sop [10,11]. It is a small semi-evergreen tree/shrub, 3-7 m tall, with irregular or crown branches. The leaves are oblong-lanceolate and pale green on both surfaces. The flowers are greenish-yellow and produced in single or short lateral clusters [13]. The petioles are green and 0.6-1.3 cm in length. The fruit of this plant is round, heart shaped, ovate or conical. It is green-yellow in colour initially, but the ripe fruit is white with the sweetly aromatic pulp also white [10]. The seeds are shiny, numerous, and blackish or dark brown in colour [14]. It is used as a medicine for a general tonic, enriches blood, relieves vomiting, cancer, vermicide, skin complaints and also used for applied wounds and ulcer [13,15,16].

The third plant used in this study is *Alstonia angustiloba* which belongs to the Apocynaceae family. The local name of this plant is *pulai* or *pulaibukit* [17-19]. This plant is used as a medicine for skin sores and gynaecological diseases in Indonesia [20]. It has rich indole alkaloids in different parts, which showed cytotoxicity against KB cells [21]. The aim of this study is to isolate and determine of these plants.

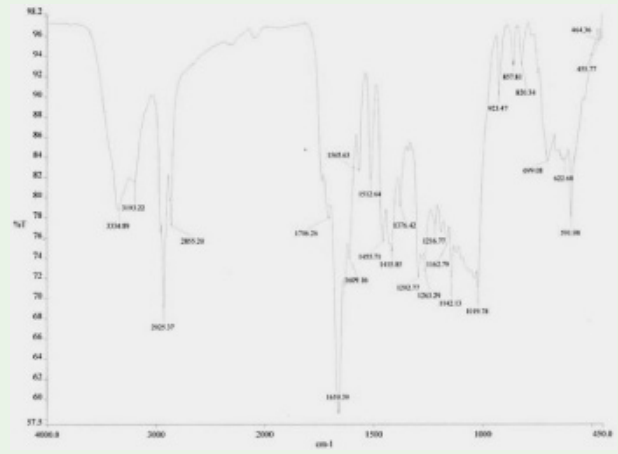

Figure 1: IR spectra of alkaloids extracted from the fruit of *M. citrifolia*.

Materials and Methods

Plant collection

The fresh ripe fruit and leaves of *M. citrifolia* were collected from Sendayan Valley, Seremban, *A. squamosa* was collected in November 2010, from Juasseh, Kuala Pilah, and *A. angustiloba* roots were collected from the herbarium of the University of Malaya. These plants were identified at the herbarium under the registration numbers KLU 22480, KLU 047368 and KLU 33380 respectively. All samples were washed under tap water and dried in an oven at 40°C for 3 days. The plant materials were then put through a grinder with a mesh size of 2 mm.

Alkaloid extracts from *M. citrifolia* fruit

This method is based on [22]. One hundred grams of the dried fruit powder was added to the mixture of ethanol-chloroform 1:3 with 2% of strong ammonia solution and refluxed for 6 hr. Extraction was conducted with 2N HCl and the extract was made alkaline with strong ammonia. The solution was extracted with chloroform and washed with distilled water. Chloroform was then evaporated until the solvent was removed at 40°C using a rotary evaporator (Heidolph WB2000, Germany). The product yield was 0.1% of original material.

Alkaloid extracts from *A. squamosa* leaves and *A. angustiloba* roots

The method is based on [23] with some modifications the modifications included varying the quantity of samples used and the incubation period of extraction. Two hundred and fifty grams of the dried powder of the parts of the plants was immersed in 100% of cold distilled methanol. The extracts were filtered with a Whatman No.1 filter paper and methanol was removed at 40°C using a rotary evaporator (Heidolph WB2000, Germany). They were then added to 5% acetic acid. The liquids were extracted with dichloromethane and the aqueous layer was basified with 10% sodium carbonate to regulate the pH to 10. Further extractions of their compounds were conducted with dichloromethane. The extracts were concentrated under reduced vacuum at 40°C

Thin layer chromatography and IR spectrometry

TLC chromatography based on the method [22]. Anthraquinones fractions of all parts of the bioactive compounds were loaded on TLC plates 60 F254 (Merck, Germany). The mobile phase dichloromethane: methanol (9:1) and spray by using Dragendroff's reagent gets the orange bands for alkaloids. All TLC plates were visualized under UV light at wavelength 245 nm and 356 nm. Then, the IR spectrum of these compounds was recorded by FTIR (Perkin Elmer spectrum 400 FT-IR, UK) at room temperature from 400 to 4000 cm^{-1} for scanning directly.

Results and Discussion

TLC results of alkaloids, five orange bands were observed for *M. citrifolia* fruit, six for *A. squamosa* and five bands for *A. angustiloba*. TLC of alkaloid extracts from the plants used in this study revealed the presence of these compounds by using Dragendroff's reagent to reveal characteristic orange bands of alkaloids. *M. citrifolia*, *A. squamosa* and *A. angustiloba* are known for their alkaloid content [7,24,25]. The functional groups of IR spectra of alkaloids extract from the fruit of *M. citrifolia*, leaves of *A. squamosa*, and the roots of *A. angustiloba* exhibited a strong O-H at band 3334.89 from the fruit of *M. citrifolia* (Figures 1, 2 and 3).

C-H stretching groups are shown at bands 2925.74 cm^{-1} and 2855.38 cm^{-1} for the fruit of *M. citrifolia*, 2852.92 cm^{-1} and 2922.89

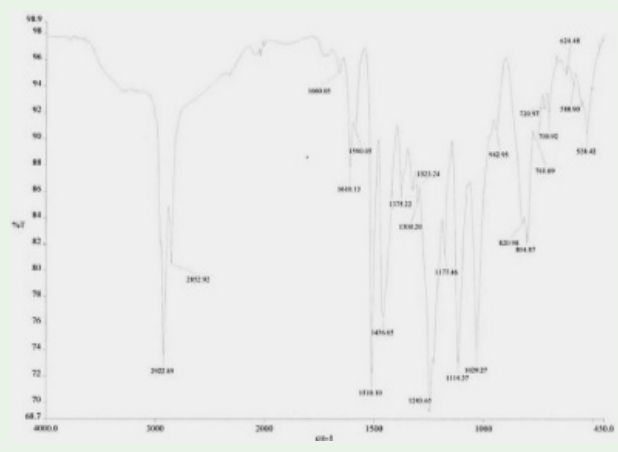

Figure 2: IR spectra of alkaloids extracted from the leaves of *A. squamosa*.

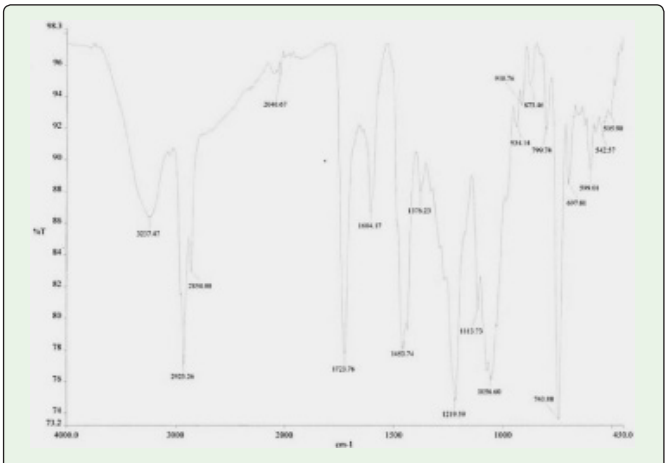

Figure 3: IR spectra of alkaloids extracted from the roots of *A. angustiloba*.

cm^{-1} for the leaves of *A. squamosa* as well as 2854.00 cm^{-1} and 2923.26 cm^{-1} for the roots *A. angustiloba*. The N-H groups showed absorption bands at 3193.22 cm^{-1} for the fruit of *M. citrifolia* and 3237.47 cm^{-1} for the root *A. angustiloba*. The C=O bond at 1660 cm^{-1} is attributed to the leaves of *A. squamosa* and 1723 cm^{-1} to the roots of *A. angustiloba*. C-H group bonds were detected at absorption bands 1565.63 cm^{-1} and 1512.64 cm^{-1} for the fruit of *M. citrifolia*, 1456.56 cm^{-1} for the leaves of *A. squamosa* and 1453.74 cm^{-1} for the roots of *A. angustiloba*. IR spectra of alkaloids extracted from the fruit of *M. citrifolia*, leaves of *A. squamosa*, and the root *A. angustiloba* exhibited a strong O-H band, N-H band, C=O bond and C-H stretching groups in these extracts. Schulz and Baranska [26] reported that alkaloids extracted from different plants have major functional groups O-H, N-H, C=O and C-H stretching groups of these compounds confirming the presence of alkaloids in these plants. In conclusion, this is the first report that studied isolation and identification of alkaloids extracts from these plants. Alkaloids extracted from these plants identified important compounds which may be used to develop biopharmaceuticals against infectious diseases with antioxidants source in future.

Acknowledgment

The authors would like to thank University of Malaya for the financial and lab facilities support for this study from IPPP grant (PV034/2011A).

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