Beni-Suef University Journal of Basic and Applied Sciences xxx (2017) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

Beni-Suef University
Journal of Basic and Applied Sciences

journal homepage: www.elsevier.com/locate/bjbas

Antimicrobial and cytotoxic comparative study of different extracts of Omani and Sudanese *Gum acacia*

Said Musallam Al Alawi, Mohammad Amzad Hossain *, Ahmed A. Abusham

School of Pharmacy, College of Pharmacy & Nursing, University of Nizwa, P.O. Box 33, Postal Code 616, Oman

ARTICLE INFO

Article history:
Received 24 February 2017
Received in revised form 22 September 2017
Accepted 21 October 2017
Available online xxxx

Keywords:
Gum acacia
Maceration
Antimicrobial activity
Cytotoxic activity
Disc diffusion method
Brine shrimp method

ABSTRACT

Gum acacia, known as *Gum Arabic* in Oman is widely grown all over the tropical countries including Gulf region. The antimicrobial and cytotoxic activities of different polarity organic extracts of Omani and Sudanese *Gum acacia* latex has been investigated in this study using maceration method. Both Omani and Sudanese *Gum acacia* latex samples were used to prepare different fractions using various organic solvents. The antimicrobial activity of different organic extracts was determined through disc diffusion method against clinically isolated bacterial strains. The isolated pathogenic bacterial strain are Gram (+) *Staphylococcus aureus* (*S. aureus*, Code No. 659), Gram (−) *Escherichia coli* (*E. coli*, Code No. 846), Gram (−) *Escherichia coli* (*E. coli*, Code No. 683) and Gram (−) *Klebsiella pneumoniae* (Code No. 684). The antibiotic levofloxacin and dimethyl sulphoxide (DMSO) were used as positive and negative controls. The cytotoxic activity of the above mentioned organic extracts was determined by brine shrimp lethality method (BSL). Both latex samples of various polarity organic extracts at different concentrations showed antimicrobial activity against the isolated human bacterial strains with an inhibition range of 0–15 mm. However, both latex samples did not reveal a significant cytotoxic activity at any concentration. In conclusion, the organic extracts from both types of *Gum acacia* (Omani and Sudanese) represent a good source of natural antibiotic for the treatment of various infectious diseases.

1. Introduction

Phyto is the Greek word for plant. There are so many groups of phytochemicals present in the plant kingdoms and they benefit humans in a variety of ways such as preventing or curing diseases. Phytochemicals are non-nutritive plant constituents that have disease preventive properties. A plant produces these bioactive constituents to protect itself from natural insults, but recent research demonstrates that many phytochemicals can protect humans as well against diseases. Fruits, vegetables and herbs have different phytochemicals that work differently to provide such protective action. The Gum Arabic (natural gum; *Gum acacia*), is related to two sub-Saharan species of the acacia tree, *Acacia senegal* and *Acacia seyal*. *Acacia senegal* (*A. senegal*) or Arabic gum tree is a well-known medicinal plant in Arabian Peninsula and in regions of Africa, in particular in Sudan. This plant belongs to the Leguminosae family. Locally, it is called Kumbat or Arabic gum (Ahmed et al., 1990). In Rajasthan (India) and in other countries, it is called Kher (Dastur, 1964). Both species are distributed mainly in tropical and subtropical region of the southern part of West Pakistan, Jaipur and Jodhpur in India and Arabian Peninsula. It is also available in Oman and grows up to 2–15 m height, with a flat or rounded crown (Maundu et al., 1999). The colour of leaves of *A. senegal* is grey-green and it has small and alternate cream coloured flowers, the seeds colour is greenish-brown (Duke, 2012). Since ancient time, *A. senegal* tree has been used for including soil fertility, provision of wood for fuel, local construction and as poles for fence posts (Fagg and Allison, 2004). Recently it was used as additive in foods, pharmaceuticals and other industries in the USA and Europe (Anderson and Weiping, 1992). Recently, Arabic gum has been approved by the US Food and Drug Administration department as food additive. Traditionally, the gum is used for the treatment of inflammation of intestinal mucosa, and externally to cover inflamed surfaces, burns and nodular leprosy. It is also in use as antitussive, astringent, and for treatment of diarrhoea, dysentery, gonorrhoea, sore throat and urinary tract ailments (Duke and Wain, 1981). In Oman, the bark and latex of Gum acacia are used traditionally for the treatment of bedsores and wounds. Known active ingredients of *Gum acacia* include neutral sugars (rhamnose, arabinose and galactose), acids (glucuronic acid and 4-methoxyglucuronic acid)

* Corresponding author at: School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P. O. Box 33, Postal Code 616, Nizwa, Oman.
E-mail address: amzad@unizwa.edu.om (M.A. Hossain).

https://doi.org/10.1016/j.bjbas.2017.10.007
2314-8535/

calcium, magnesium, potassium and sodium (Leung, 1980). It also contains various secondary metabolic compounds such as flavone, catechin, polyphenols, tannins, chalcones, alkaloids and flavonoids (Majekodunmi et al., 2006). Most of the chemical constituents/ingredients in the gum are quite safe (Dondain and Phillips, 1999). Pharmacological studies on this plant revealed that *Gum acacia* is used to clean urinary bladder (Ljubuncic et al., 2005) and alleviate adverse effects of chronic renal failure (Ali et al., 2008). There are several scientific evidences in the literature on antioxidant and cytotoxic activities of the crude extracts of *Gum acacia* (Elegami et al., 2001). The literature is scant with regard to antimicrobial and cytotoxic activities of *Gum acacia* (Elegami et al., 2001; Singh et al., 2015; Hu et al., 2016). Furthermore, there are no studies available in the literature on Omani *Gum acacia*, although Sudanese gum was fairly studied. Therefore, in this study, we intended to prepare different organic extracts of latex of Omani and Sudanese *Gum acacia* by using different solvents with increasing polarity to determine and compare their antimicrobial and cytotoxic activities. Although antibacterial and cytotoxic activities of *Gum acacia* have been reported (Vijayasanthi et al., 2012; Otto et al., 2014), to the best of our knowledge, this is the first report of comparative study of antibacterial and cytotoxic activities for two types of *Gum acacia*.

2. Materials and methods

2.1. Materials

The antibiotic levofloxacin (Purity 99.87%) and dimethyl sulphoxide (DMSO, purity 98%) used in this experiment were obtained from Sigma–Aldrich®, Germany. All experimental solvents such as methanol (Purity 99.76%), *n*-hexane (Purity 95.23%), *n*-butanol (Purity 98.56%), chloroform (Purity 99.87%), ethyl acetate (Purity 95.01%) and acetone (Purity 96.33%) were obtained from Merck® (Darmstadt, Germany). The purified distilled water was prepared in our laboratory. All other chemicals were of analytical grade. Filter paper and discs were from Whatmann®. All glassware like beakers, conical flasks, round bottom flasks, cylinders, test tubes etc., were from Pyrex®, UK. The rotary evaporator was obtained from Yamato Company®, Japan. The Incubator for was obtained from VWR Company®, Germany and the oven is the product of Memmert®, Germany.

2.2. Microorganism

The clinically isolated human pathogenic bacterial strains including both Gram (+) *Staphylococcus aureus* (*S. aureus*, Code No. 659), Gram (−) *Escherichia coli* (*E. coli*, Code No. 846), Gram (−) *Escherichia coli* (*E. coli*, Code No. 683) and Gram (−) *Klebsiella pneumoniae* (*K. pneumonia* Code No. 684) were collected locally on February 23, 2016 from Nizwa Hospital, Nizwa, Oman, a reference secondary health care hospital. Patients' names and medical record numbers of the isolates are anonymous.

2.3. Sample collection

The latex of Omani *Gum acacia* samples was collected from Salalah, Sultanate of Oman and the Sudanese latex was collected from Sudan through one of the authors on February 15, 2016. Both the samples were stored at 4 °C to avoid degradation of the chemical ingredients.

2.4. Sample preparation and extraction

The collected latex samples (each = 75.5 g) were suspended in water (150 ml) separately at ambient temperature (25 °C) and the pH 7. The whole mixture was transferred to a separatory funnel for fractionation. Different polarities of organic solvents were used including *n*-hexane, chloroform, ethyl acetate and *n*-butanol for this process. The process was repeated two times. Each solvent from the isolated fractions from the latex of Omani and Sudanese *Gum acacia* was evaporated by a rotary evaporator at 22 °C at high vacuum reduced pressure. After evaporation of Omani latex, it revealed *n*-hexane (15. 97 g, yield 21.1%), chloroform (11.13 g, yield 14.74%), ethyl acetate (7.36 g, yield 9.74%) and *n*-butanol (4.63 g, yield 6.13%), and water (35.62 gm, yield 47.17%) respectively. Similarly, after evaporation of Sudanese latex, it revealed *n*-hexane (5.35 gm, yield 7.08%), chloroform (10.65 g, yield 14.10%), ethyl acetate (13.54 g, yield 17.93%), *n*-butanol (1.03 g, yield 1.36%), and water (34.93 g, yield 46.26%) respectively (Almatani et al., 2015). All fractions from Omani and Sudanese *Gum acacia* were used for the determination of antimicrobial and cytotoxic activities.

2.5. Antimicrobial activity

The antimicrobial activity of different extracts isolated from Omani and Sudanese *Gum acacia* was determined through disc diffusion method with modification (Al-Jadidi and Hossain, 2015). Each of the prepared organic extracts was used to test the antimicrobial activity against one Gram (+) pathogen: *Staphylococcus aureus* (*S. aureus*, Code No. 659) and three Gram (−) pathogens: *Escherichia coli* (*E. coli*, Code No. 846), *Escherichia coli* (*E. coli*, Code No. 683) and *Klebsiella pneumoniae* (*K. pneumonia* Code No. 684). The antibiotic levofloxacin in a concentration of 1 mg/ml was used as a positive control, and DMSO was used as a negative control. A 6 mm diameter filter paper discs was used in this experiment and the discs were made from Whatmann® filter paper by using a punch machine. Four different extract concentrations of 2, 1, 0.5 and 0.25 mg/ml were used for the determination of antimicrobial activity. The sterile filter paper discs were soaked with each organic extract at different concentrations and placed on the inoculated agar. All the plates were incubated at 37 °C for 24 h. The evaluation for antibacterial activity was measured as the diameter of zones of inhibition against the tested bacteria. Activity index for each extract was calculated by using the following formula:

$$\text{Activity index (AI)} = \text{Inhibition zone of the sample} / \text{Inhibition zone of the standard}$$

2.6. Determination of minimum inhibitory concentrations (MIC) of the organic extracts

A plot of the diameter of the zones of inhibition against log concentration of the dilutions was done and a suitable curve drawn from the plots for each extract. Extrapolation of the curve was done to determine the log of MIC. From this log the MIC was calculated as the antilog (Otto et al., 2014).

2.7. Cytotoxic activity

The cytotoxic activity of the prepared organic extracts at different concentrations of Omani and Sudanese *Gum acacia* latex was determined by brine shrimp lethality method (Weli, et al., 2014). The brine shrimp eggs were hatched in a covered chamber of duo compartment (plastic container with sea water) for 24 h. After hatching, the active nauplii were separated from the eggs to another compartment. The active nauplii were used to test cytotoxic activity of *Gum acacia*. Six different extract concentrations of 10, 100, 250, 500, 750 and 1000 μg/ml were prepared using distilled water. One ml from each test solutions was added to a

Please cite this article in press as: Alawi, S.M.A., et al. Antimicrobial and cytotoxic comparative study of different extracts of Omani and Sudanese *Gum acacia*. Beni-Suef Univ. J. Basic Appl. Sci. (2017), https://doi.org/10.1016/j.bjbas.2017.10.007