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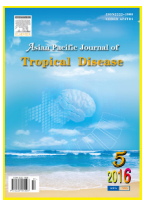

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Determination of the total phenols, flavonoids and antimicrobial activity of the crude extracts from locally grown neem stems

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ABSTRACT

Objective: To prepare the crude extracts from the stems of neem with maceration extractor and determine their total phenolics, flavonoids contents and antimicrobial activity by established methods.

Methods: The dry stem samples were used to prepare different crude extracts using maceration method. The total phenolics and total flavonoids contents were determined through Folin-Ciocalteu's reagent and $AlCl_3$ methods. The antimicrobial activity of the crude extracts from the local neem was determined by disc diffusion method against one Gram-positive *Staphylococcus aureus* and three Grams-negative *Escherichia coli*, *Pseudomonas aeruginosa* and vulgaris pathogenic bacterial strains. The standard amoxicillin was used as a positive control for the antimicrobial study.

Results: The results of the total phenolics content of the crude extracts from different stems ranged from 20.80 mg/100 g to 107.29 mg/100 g of the powder crude extracts. The total flavonoids content of different crude extracts from powder stems of neem ranged from 136.50 mg/100 g to 484.50 mg/100 g of the powder samples. The antimicrobial activity of the crude extracts from different stems of neem at different concentrations against four bacterial strains didn't give any activity.

Conclusions: Thanks to the contents of the total phenolics and flavonoids, the crude extracts could be used as medicines for different aliments.

1. Introduction

Azadirachta indica A. Juss (*A. indica*) is a kind of tree belonging to Meliaceae family. It comes from Pakistan, India and Thailand[1]. Its English name is neem. Locally it is called "alshurisha". Neem tree grows very fast. The branches are wide and spreading. Leaves are mixed of both young and mature. The mature leaves are bright green and the young leaves are reddish to purplish color. Typical leaves consist of three main parts: leaf base, petiole and lamina. Leaves are attached to the stem by their leaf bases and may bear two lateral small leaves like structures called stipules[2].

Neem is used as traditional medicine in Indian culture for the treatment of different aliments. Neem is one of the main sources of many therapeutic agents[3]. In Thai traditional medicine system, its leaves and flowers are used as a tonic for the treatment of fever[4]. The leaf and seed oils are very important medicines used for the treatment of symptoms of psoriasis and relives itching[5]. Similarly, the fruits are used as an anthelmintic for the treatment of hemorrhoids and abnormal urination[6]. Traditionally, this tree was preferred over others because it produces fewer allergies and side effects[7].

Medicinally it is used widely as alternative therapeutic tools for the prevention or treatment of many diseases[8]. Each part of neem tree has several medicinal values to treat a wide range of human disorders such as antiseptic, diuretic, cough, nausea, vomiting, fever and peptic ulcer[9]. The most common use of this plant's juice is for gastrointestinal diseases[9]. Traditionally, Indian people used the leaves for the treatment of chicken pox sleep[10].

There is much extensive work by scientists on the analysis of chemical constitutions of the crude extracts neem. Many bioactive components have been isolated and identified from neem crude extracts such as azadirachtin, salannin, meliantriaol and nimbin[11]. Among them the most active ingredient is reported as azadirachtin[11]. These chemical constitutions belong to the classes: beta-sitosterol, stigmasterol and limonoids[11,12]. Also, the other tricyclic and tetracyclic compounds have been isolated from this plant such as sulphides, flavonol glycosides. nimaton, quercetin,

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myrecetin and kaempferol[12].

Traditionally, Omanis use the whole plant for the treatment of fever, to kill worm and so on[12]. The literature search reveals that no scientific data on the total phenolics, flavonoids and antimicrobial activity are available in Oman. Therefore, the main objectives of this present study is to prepare crude extracts using different polarities of solvents by maceration method and determine their total phenolics, flavonoids and antimicrobial activity from the crude extracts of neem stems collected from Muscat.

2. Materials and methods

2.1. Materials and chemicals

The solvents acetone, butanol, chloroform, ethyl acetate, methanol, ethanol, hexane dichloromethane and dimethyl sulphoxide (DMSO) were collected from Sigma-Aldrich Company, Germany. The standard gallic acid and qurecetin for the determination of the total phenolics and flavonoids were collected from Sigma Company Ltd, Germany. Sodium hydroxide and aluminum chloride were obtained from Philip Harris, England. Folin-Ciocalteu's reagent (FCR) and sodium nitrate for the total phenolics were obtained from Scharlau, Spain. UV-visible spectrophotometer (UV-visible) was from Jasco Company, Japan.

2.2. Bacterial strains

The employed bacterial strains against different crude extracts of neem such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Proteus vulgaris* (*P. vulgaris*) were collected from the Department of Biological Sciences, College of Arts and Sciences, University of Nizwa, Sultanate of Oman.

2.3. Plant sample

The stem samples were collected from Al-Amerat, Muscat, Sultanate of Oman. The samples were harvested on February 8, 2014 at 3 pm. The collected samples were transported to the Natural Product Lab, School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, Sultanate of Oman. The plant was identified by botanists and the specimen number (006) was deposited in the lab.

2.4. Preparations of samples

The stem samples were washed to remove the unwanted substances. Then, the samples were cut into small pieces and dried at room temperature for three days. The dried stem samples were powdered by a grinding machine.

2.5. Extraction procedure

The stem powder (150 g) were taken in a beaker and 250 mL of methanol was added. The mixture was kept for 3 days. The sample was filtered by using a Bruckner funnel. After filtration, the filtrate was taken in a round bottom flask and evaporated by using a rotary evaporator. After evaporation, the crude extract was dissolved in water (150 mL) and shaken until it was dissolved. The dissolved mixture was transferred into a separatory funnel and solvents with different polarities were added twice with increasing polarities such as hexane, chloroform, ethyl acetate and butanol (30 mL, and 20 mL). After the extraction procedure was finished, the solvent was evaporated to get crude extracts with different polarities.

2.6. Determination of the total phenolics content

2.6.1. Preparation of reagents

FCR (10 mL) was taken in a 100-milliliter volumetric flask with 90 mL of water. Three grams of sodium carbonate was taken in a 50-milliliter volumetric flask with 50 mL of water.

2.6.2. Procedure

The prepared crude extracts from the stems of neem (4 mg) were taken in separate test tubes and 4 mL of methanol was added. A total of 200 μL of solutions from each crude extract was taken in a test tube and 1.5 mL of FCR was added and then kept in dark place for 5 min. After dark, 1.5 mL of sodium carbonate was added to the same solution and mixed by hand. All the tubes were kept at dark place for 2 h. Finally, the absorbance was recorded by using UV-visible at the wave length of 760 nm[13].

2.7. Determination of the total flavonoids content

The prepared crude extracts (4 mg) were taken in separate test tubes and 4 mL of methanol was added. Then, 0.25 mL of each crude solution was taken in a test tube and 1.25 mL of water and 75 μL of sodium nitrate were added. The whole mixture was kept for 6 min and 150 μL of aluminum chloride was added to react for 5 min in the dark place. Finally, 0.5 mL of sodium hydroxide and 0.275 mL of water were added to it. The absorbance was measured by UV-visible at the wave length of 450 nm[14].

2.8. Antimicrobial activity test

Each crude extracts from the stems of neem (4 mg) was taken in a volumetric flask and 4 mL of DMSO was added[15]. Different concentrations such as 2.00 mg/mL, 1.00 mg/mL, 0.50 mg/mL and 0.25 mg/mL were prepared by serial dilution technique. Amoxicillin (3 mg) was used as a standard by adding 3 mL of DMSO. Each prepared concentrations of each crude extracts was tested for its antimicrobial activity against one Gram-positive bacteria *S. aureus* and three Gram-negative bacteria *E. coli, P. aeruginosa* and *P. vulgaris* on MacConkey agar plate using disc diffusion method. Whatmann filter paper No. 41 sterile filter paper discs with a diameter of 6 mm were impregnated with each crude extract and placed on the inoculated agar. The concentration of amoxicillin standard used for this study was 1 mg/mL. All the plates were incubated at 37 °C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones against the tested bacteria. Each method in this experiment was replicated three times.

3. Results

The powdered stem samples were used for the extraction with methanol by maceration method for 3 days. After completing extraction, methanol was evaporated by a rotary evaporator and defatted with water. Then, different solvents such as hexane, chloroform, ethyl acetate and butanol were used for extraction with increasing polarities.