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Investigation of polyphenolic compounds, cytotoxic and antimicrobial activities of *Callistemon comboynensis* leaves

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ABSTRACT

Phytochemical and biological activity of ethanolic extract of *Callistemon comboynensis* leaves resulted in identification of six known polyphenolic compounds identified as Gallic acid, Ellagic acid, Kaempferol 3-*O*-α-L-rhamnopyranoside, Methoxy ellagic acid, Quercetin and Kaempferol. The ethanolic extract showed moderate cytotoxic activity against the tested cell line, in addition to significant antibacterial activity against six potential pathogenic bacteria isolated from bottled mineral water.

INTRODUCTION

Callistemon is a well-known genus, it is known in folk medicine for its various beneficial biological activities. *Callistemon* (commonly named bottle brush) comprises about 34 species belong to the family Myrtaceae, which are widely cultivated and much used as ornamental shrubs in warm countries[1-7]. Many phenolic compounds were isolated from different species of this genus have been identified[8-11]. *Callistemon comboynensis* Cheel, also known as Cliff Bottlebrush, is a shrub native to the states of Queensland and New South Wales in Australia and the species grows up to 2 meters in height. Nothing has been reported concerning the phytochemical and biological studies of *C. comboynensis* except investigation of the antimicrobial activities of volatile oil of its leaves[12]. The present work is concerned with investigation of polyphenolic compounds in *Callistemon comboynensis* leaves and evaluation of the anticancer activity of the total ethanolic extract of its leaves against the P388 cell leukemia, and explores the antimicrobial activity against gram positive and gram negative bacterial strains isolated from bottled mineral water as potentially pathogenic bacteria.

MATERIALS AND METHODS

Plant material

Identification of the plant was confirmed by Dr. Trease Labeb, senior specialized of plant taxonomy, Orman Garden, Giza, Egypt as well as by comparison with reference herbarium specimens.

Extraction and isolation

A powdered, air-dried leaf of *C. comboynensis* (500 g) was exhaustively extracted with hot 80% EtOH (4 × 3 L), under reflux. The dry residue obtained (80 g) was extracted with chloroform (3 × 1 L). The aqueous residue was fractionated on a polyamide column (Ø 5.5 × 120 cm) and was eluted with water followed by water/methanol mixtures of decreasing polarities to afford several fractions. Those fractions were concen-

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trated under vacuum and purified on Sephadex LH-20 column and or Cellulose columns using different eluting systems.

Cytotoxic activity against P388 Leukemia cells

The cell line employed in the present investigation (P388 leukemia cells) was obtained from the American Type Culture Collection (Rockville, MD). This cell line was cultured in Fisher's medium containing 10% horse inactivated serum at 37°C in an atmosphere of 5% CO2 (100% humidity). The cultured cells were treated in triplicate with various concentrations (0.5-100 ug/ml) of the extracted plant dissolved in 100 µl DMSO followed by shaking. The culture cells were incubated for 18 h at 37 °C in a humidified atmosphere of 5% CO_2. The cell concentration was determined by counting the P388 cells in a hemocytometer[13].

Antibacterial activity against gram positive and gram negative bacteria

The screening of the ethanolic extract of the plant leaves for anti-bacterial activity was performed using the disc diffusion method against three gram positive bacterial strains *Bacillus* sp. HM03, *Bacillus* sp. HM07, and *Exiguobacterium* sp. HM04 with accession numbers JQ396176, JQ396180 and JQ396177, respectively and three gram negative bacterial strains *Acinetobacter* sp. HM01, *Pseudomonas* sp. HM05, and *Pseudomonas* sp. HM06 with the accession numbers JQ396174, JQ396178 and JQ396179, respectively were provided from Dr. Hamdy A. Hassan Environmental Biotechnology Department, GEBRI Menoufiya Uni., Egypt. The disc diffusion test was performed using the standard procedure by the National Committee for Clinical Laboratory Standards (NCCLS). The inoculums suspension of each bacterial strain was swabbed on the entire surface of Mueller-Hinton agar (MHA, Biokar-diagnostics). Sterile 6-mm filter paper discs were aseptically placed on MHA surfaces and crude ethanol extracts were immediately added to discs in volumes of 10 µl. The plates were left at ambient temperature for 15 min to allow excess prediffusion of extracts prior to incubation at 37°C for 24 h. Diameters of inhibition zones were measured. Each experiment was done in triplicates. A microbial susceptibility control test was performed with some antibiotic discs with different targets: Tetracycline (30 µg), Chloram-phenicol (30 µg), Ampicillin (and 30 µg). Which were used as experimental positive control while ethanol solvent was used as negative control.

Instruments and materials

The UV analysis for pure samples was recorded on a Shimadzu UV 240 spectrophotometer, separately as solutions in methanol and with different diagnostic UV shift reagents. Fractionation of the extracts was done by column chromatography using polyamide 6S (Riedel-De Han Ag, Seelze Hannover, Germany) and Sephadex (Fluka,Switzerland), isolation and purification of compounds was done on either cellulose LH-20 (Pharmacia, Uppsala,Sweden) or Sephadex columns of different dimensions and eluted with different solvent systems. Separation processes were followed up by 2D-PC and CoPC using Whatmann No. 1 paper with (S1) *n*-BuOH-AcOH-H_2O(BAW) (4:1:5, top layer) and (S2) 15% aqueous AcOH as solvent systems. NMR (^{1}H- and ^{13}C NMR) spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian Mercury 300. The δ-values are reported as ppm and *J*-values are in Hz. Negative ESI MS Spectrometer: Finnigan LCQ-deca, Finnigan.

RESULTS AND DISCUSSION

Investigation of polyphenolic contents

Ethanolic extract of *Callistemon comboynensis* leaves was fractionated on a polyamide column followed by successive separation on cellulose and sephadex LH-20 columns using different eluting systems yielded six compounds. The isolated pure compounds were identified on the basis of acid hydrolysis, comparative PC, UV, ESI-MS, ^{1}H- and ^{13}C-NMR spectroscopic analysis and comparing with previous reported data[8-11&13-17]. The known isolated compounds are identified as Gallic acid, Ellagic acid, Kaempferol 3-*O*-α-L-rhamnopyranoside, Methoxy ellagic acid, Quercetin and Kaempferol.

Biological assay against P388 leukemia cells

The biological assay of the ethanol extract of the *Callistemon comboynensis* leaves showed that it has moderate activity against P388 leukemia cells (ED50=

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37.6 µg/ml) whereby the results expressed as the dose that inhibits 50% control growth after the incubation period (ED50), compounds having ED50 µg/ml <20 were considered active. That activity may be revealed to its polyphenolic contents where phenolic compounds are believed to have chemo preventive and suppressive activities against cancer cells by inhibition of metabolic enzymes involved in the activation of potential carcinogens or arresting the cell cycle[18].

Antibacterial assay

The antibacterial activities of the extracts in terms of minimum inhibitory concentrations (MIC) and diameters of inhibition zones are reported in TABLE 1, 2. The ethanolic extract of *callistemon comboynensis* leaves found to be prominently active against the tested micro-organisms at the concentration 30 ug/mL (MIC). The ethanolic total crude extract of *Callistemon comboynensis* showed reasonable, comparable inhibitory activity against the Gram positive organisms; whereas there was moderate activity against Gram negative bacteria. The bioactivities of the extract which elicited antibacterial activity appeared to have preferential and specific activity against Gram positive bacteria.

TABLE 1 : MIC of ethanol extract of *Callistemon comboynensis* leaves

Name of bacteria	Growth in nutrient agar containing different concentrations of extract (µg/ml)						
	0	10	20	30	40	50	60
Bacillus sp. HM03	+	+	+	-	-	-	-
Bacillus sp. HM07	+	+	+	-	-	-	-
Exiguobacterium sp. HM04	+	+	+	-	-	-	-
Acinetobacter sp. HM01	+	+	+	-	-	-	-
Pseudomonas sp. HM05	+	+	-	-	-	-	-
Pseudomonas sp. HM06	+	+	+	-	-	-	-

All determinations were done in triplicates. '0'Control (without extract);'+'Growth;'-'No growth

TABLE 2 : Diameters of Inhibition Zones produced by the ethanol extract of *Callistemon comboynensis* leaves, Tetracycline, Chloram-phenicol and Ampicillin

Name of bacteria	Ethanol extract (30 µg)	Tetracycline (30 µg)	Chloram-phenicol (30 µg)	Ampicillin (30 µg)
Bacillus sp. HM03	7	7	7	7
Bacillus sp. HM07	7.3	7	8	9
Exiguobacterium sp. HM04	8	8	7	9
Acinetobacter sp. HM01	6.5	8.3	8	8
Pseudomonas sp. HM05	6.3	7	8	9
Pseudomonas sp. HM06	6.2	8	9	7

Inhibition zone, including the diameter of the filter paper disc (5 mm); Tetracycline, Chloram-phenicol and Ampicillin were used as positive control

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