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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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. 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=...
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\textsuperscript{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 µg/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**

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Growth in nutrient agar containing different concentrations of extract (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><em>Bacillus</em> sp. HM03</td>
<td>+</td>
</tr>
<tr>
<td><em>Bacillus</em> sp. HM07</td>
<td>+</td>
</tr>
<tr>
<td><em>Exiguobacterium</em> sp. HM04</td>
<td>+</td>
</tr>
<tr>
<td><em>Acinetobacter</em> sp. HM01</td>
<td>+</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. HM05</td>
<td>+</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. HM06</td>
<td>+</td>
</tr>
</tbody>
</table>

All determinations were done in triplicates. "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**

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Ethanol extract (30 µg)</th>
<th>Tetracycline (30 µg)</th>
<th>Chloram-phenicol (30 µg)</th>
<th>Ampicillin (30 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus</em> sp. HM03</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><em>Bacillus</em> sp. HM07</td>
<td>7.3</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td><em>Exiguobacterium</em> sp. HM04</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td><em>Acinetobacter</em> sp. HM01</td>
<td>6.5</td>
<td>8.3</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. HM05</td>
<td>6.3</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. HM06</td>
<td>6.2</td>
<td>8</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

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

**REFERENCES**

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