Evaluation of antioxidant activity of the methanolic leaf extract of *Clausena excavata* Burm. f. (Rutaceae) using the lipid peroxidation model

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**ABSTRACT**

*Clausena excavata* Burm. f. (Rutaceae) has long been associated with medicinal benefits in folk medicine, particularly in the treatment of cancer and its related disorders in the eastern region of Thailand. The present study evaluates the antioxidant activity of the methanolic leaf extract of *C. excavata* using the lipid peroxidation model. The results indicated that the methanolic leaf extract showed marked antioxidant activity (IC₅₀ = 201.3 µg/ml), whereas the aqueous leaf extract showed moderate antioxidant activity (IC₅₀ = 450.6 µg/ml). In conclusion, the antioxidant activity of the methanolic leaf extract of *C. excavata* was comparable with that of propyl gallate, which is used as a standard drug, thus confirming that it might be a good source of antioxidants. The extended degree of antioxidant activity could be attributable to the presence of flavonols, gallic acid, and angelicin, which are known to be antioxidants.

**Keywords:** Free radical, MDA, Propyl gallate, TBARS, Rutaceae.

**INTRODUCTION**

Ethnobotanical screening of species of the Rutaceae family in several continents has shown that they have important medicinal properties. *Clausena excavata* is a wild shrub of the Rutaceae family that is distributed in South Asia. It is also cultivated in some areas and grows up to 1.5 m high. It has been used in folk medicine for the treatment of cancer and several disorders in the eastern parts of Thailand.¹⁻⁴ Its leaves and stem are also used to treat colic, cough, headache,⁵ rhinitis, sores, wounds, and yaws, and it is used for detoxification in some countries.⁶ The leaves are particularly used in traditional medicine to cure cold, abdominal pain, malaria,⁷ dysentery, and anti-platelet aggregation.⁸ The plant is also reported to have insecticidal, tonic, and vermifugal properties.⁹ *C. excavata* has been reported to contain carbozole alkaloids, coumarins, limnoids,⁴ etc. The sap of the leaves is applied on the affected area to treat all kinds of muscular pain.⁹ A decoction of the root is given to patients with malaria; an infusion of the stem is given in patients with colic. The leaves and stem bark are also used locally as a diuretic, tonic, astringent, and antinociceptive agent.⁹ The coumarins derived from the leaves have been found to have inhibitory effects on tumor progression.⁹⁻¹⁰ Several pharmacologically active constituents of plants belonging to the family Rutaceae have been identified—phenylpropanoids,⁹ methoxyflavones,¹⁰ furoquinoline alkaloids,¹¹ coumarins,¹² and acridone alkaloids.¹³ However, the chemical components of species of this family are yet to be fully elucidated. We chose to study the phytotoxicological properties of extracts of *C. excavata* because it is known to have antioxidant properties. Cellular damage arising from reactive oxygen species (ROS) has been shown to play a part in the development and

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pathophysiology of neurodegenerative disorders (e.g., Alzheimer disease, Parkinson disease, multiple sclerosis, and Down syndrome), inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer. We therefore focused our investigation on the antioxidant potential of *C. excavata*.

The aim of the present investigation was to separate, characterize, and identify the major chemical constituents of *C. excavata* and to explore the antioxidant potential of its methanolic extracts and isolated constituents. The thiobarbituric acid reactive species (TBARS) assay was performed using standard methods with melondialdehyde (MDA) equivalents derived from tetraethoxypropane. MDA and other aldehydes have been identified as products of lipid peroxidation that react with TBA to yield a pink product that shows absorption at 532 nm.

**MATERIALS AND METHODS**

**Plant material**

Leaves of *C. excavata* were collected from the northern regions of Malaya in June-July 2005, and their identity was further authenticated by Bowen, from the Department of Pharmacognosy, University of Sunderland, and a voucher specimen sample was deposited in the herbarium. The collected plant material were dried well under shade and powdered using electric blender.

**Soxhlet extraction**

The powdered leaf material (100 g) was successively extracted with petroleum ether, hexane, chloroform, dichloromethane, ethanol, and water. The volume of the solvent used in each case was 500 ml. Before extraction with each solvent, the powdered material was air dried and weighed. The largest quantity of extract was obtained with methanol. Further fractionation of the extracts was therefore carried out on the methanolic leaf extract by high-performance liquid chromatography.

**Preliminary phytochemical screening**

The different qualitative tests can be performed for establishing profile of the plant extracts for its chemical composition. The freshly prepared extracts were subjected to standard phytochemical analysis to test for the presence of the phytoconstituents like tannins, saponins, alkaloids, carbohydrates, glycosides, phenolics, flavonoids and terpenoids. Chemical tests were carried out on methanol and aqueous extracts of the powdered specimens using standard procedures for the detection of saponins, alkaloids (Mayer’s reagent and Dragendorff’s reagent test), carbohydrates (Molish’s and Benedict’s test), glycosides (Borntrager’s and Legal’s tests), phenolics and tannins (Ferric chloride and Lead acetate test), flavonoids (alkaline reagent test), terpenoids (Salkowski test and Trichloro acetic acid test) to identify the phytochemical constituents.

**Chemicals**

1 % Thiobarbituric Acid (TBA), Propylgallate (0.1 mM), ascorbic acid (1 mM), FeCl₃ (1 mM), 2 % butylated hydroxy toluene (BHT) in ethanol, n-butanol (Fisher Scientific), Bovine brain extract (Sigma product: B 3635), HCL (25% Hydrochloric acid), Glass balls (about 2mm diameter) were used.

**Preparation of liposomes and extracts**

Brain bovine extract (50 mg; B 3635, Sigma) was placed in a clean universal container with about 7 glass balls (diameter, 2 mm) and 10 ml of phosphate-buffered saline (PBS), and sonicated in an ice-water bath until a homogenous and milky lipid suspension was obtained. The methanolic extract (50 mg) was dissolved in 10 ml of methanol and filtered; the filtrate was used for the assay.

**Procedure for the assay**

The liposomes were prepared as a 5 mg/mL suspension of bovine brain extract (Sigma) in PBS and stored at –80°C. Ascorbic acid and ferric chloride aqueous solutions (1 mM) were freshly prepared before each test. Seven concentrations of methanolic extract were prepared (5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.07861 mg/ml). Four replicates were carried out for each concentration of the methanolic extract and without the methanolic extract.

Blank: Distilled water (0.4 mL) and 0.5 mL of PBS were added in the first row of tubes.

Free reaction mixture: Liposomes (0.2 mL), 0.5 mL PBS, 0.1 mL FeCl₃ solution, and 0.1 mL of ascorbic acid solution were added in the second row of tubes. The positive control tubes(4) contained 0.1 mL propyl gallate, 0.2 mL liposomes, 0.5 mL PBS, 0.1 mL FeCl₃ solution and 0.1 mL ascorbic acid solution, resulting in 68 tubes (Sterilin). Distilled water is used as blank whereas Propyl gallate is used as standard compound. The tubes were
heated in a water bath (Gallenkamp) at 85–90°C for 30 min, and after they had cooled, 2.5 mL of n-butanol was added. They were shaken individually with a vortex mixer (Gallenkamp) and centrifuged at 3500 rpm (MISTRAL-3000i) at room temperature for 10 min. The top n-butanol layer (2.5 mL) from each tube was transferred to a cuvette, and its absorbance was measured with a UNICAM UV/VIS spectrophotometer operating at 532 nm, using n-butanol as the internal standard.[17–18]

The data were processed with the aid of Microsoft Excel 2000, and the percentage inhibition of each compound at a given concentration was calculated. The IC$_{50}$ values were obtained from LOWESS analysis of percentage inhibition of peroxidation versus the logarithmic concentration of each inhibitor, using PRISM GraphPad. The results are shown in Table 2.

**RESULTS AND DISCUSSION**

Free radicals such as the superoxide radical, hydroxy radical, peroxyl radical, and singlet oxygen have been implicated in the pathophysiology of many diseases. Herbal drugs containing radical scavengers are gaining importance for the treatment of such diseases. Many plants exhibit efficient antioxidant properties attributable to their phenolic constituents.[19–22]

Our results indicated that the methanolic leaf extract of *C. excavata* showed marked antioxidant activity (Table 2), whereas the aqueous leaf extract of *C. excavata* showed moderate antioxidant activity. The IC$_{50}$ value (Fig. 1) of the methanolic leaf extract was found to be 201.3 µg/ml, whereas the IC$_{50}$ value for the aqueous extract of *C. excavata* was found to be 450.6 µg/ml. The methanolic leaf extract was used for the preliminary phytochemical screening to identify secondary metabolites/active constituents. Phenols, flavonoids, and tannins were identified in the extracts (Table 1). The extended degree of antioxidant activity displayed by the methanolic extract could be attributed to the presence of flavonoids,[19] gallic acid,[20–21] and angelicin,[22] which are known to be antioxidants.

Our findings therefore indicate that the methanolic extract of leaves of *C. excavata* showed significant antioxidant activity. This warrants further investigation to identify the active constituents accountable for its antioxidant activity.

<table>
<thead>
<tr>
<th>S r. No.</th>
<th>Plant</th>
<th>Part of the plant</th>
<th>Solvent for extraction</th>
<th>S</th>
<th>A</th>
<th>F</th>
<th>P</th>
<th>G</th>
<th>Sa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Clausena excavata</em></td>
<td>Leaves</td>
<td>Methanol</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td><em>Clausena excavata</em></td>
<td>Leaves</td>
<td>Methanol</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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</table>

S: Steroids; A: Alkaloids; F: Flavonoids; P: Phenolics; G: Glycosides; Sa: Saponins; +: Positive; –: Negative

**Table 1. Phytochemical test results for secondary metabolites.**

**Table 2. Antioxidant activity according to the lipid peroxidation assay.**

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Concentration (mM)</th>
<th>Absorbance at 532 nm (n = 4)</th>
<th>Mean</th>
<th>S.D</th>
<th>% R.S.D</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
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<td>0.0880</td>
<td>0.0910</td>
<td>0.1040</td>
<td>0.0828</td>
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<tr>
<td>FRM</td>
<td></td>
<td>0.2810</td>
<td>0.2930</td>
<td>0.2660</td>
<td>0.2630</td>
<td>0.2758</td>
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<tr>
<td>Propyl gallate</td>
<td>0.1000</td>
<td>0.1690</td>
<td>0.1210</td>
<td>0.1810</td>
<td>0.1740</td>
<td>0.1500</td>
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<tr>
<td>Substance test</td>
<td>5.0000</td>
<td>18.7041</td>
<td>0.1270</td>
<td>0.1740</td>
<td>0.1500</td>
<td>0.1740</td>
</tr>
<tr>
<td>Substance alone</td>
<td>5.0000</td>
<td>18.7041</td>
<td>0.0020</td>
<td>0.0040</td>
<td>0.0010</td>
<td>0.0010</td>
</tr>
<tr>
<td>Substance test</td>
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<td>9.3521</td>
<td>0.1980</td>
<td>0.1750</td>
<td>0.1860</td>
<td>0.1920</td>
</tr>
<tr>
<td>Substance alone</td>
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<td>9.3521</td>
<td>0.0030</td>
<td>0.0760</td>
<td>0.0070</td>
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<tr>
<td>Substance test</td>
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<td>4.6760</td>
<td>0.2180</td>
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<tr>
<td>Substance alone</td>
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<td>0.0810</td>
<td>0.0600</td>
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<tr>
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<td>0.3140</td>
<td>0.1560</td>
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<td>0.2350</td>
</tr>
<tr>
<td>Substance alone</td>
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<td>2.3380</td>
<td>0.0910</td>
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<td>0.2291</td>
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<tr>
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<td>0.0295</td>
<td>0.0349</td>
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<td>Substance test</td>
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<td>0.5845</td>
<td>0.3139</td>
<td>0.3160</td>
<td>0.2600</td>
<td>0.2350</td>
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<tr>
<td>Substance alone</td>
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<td>0.5845</td>
<td>0.0191</td>
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<td>0.0298</td>
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<td>0.2420</td>
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<td>Substance alone</td>
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<td>0.0093</td>
<td>0.0070</td>
<td>0.0111</td>
<td>0.0150</td>
</tr>
</tbody>
</table>

S.D: Standard Deviation, R.S.D: Relative Standard Deviation

Substance test: With methanolic extract concentration

Substance alone: Without methanolic extract concentration
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**COMPETING INTERESTS**

The authors declare that they have no competing interests associated with this study.

**REFERENCES**


