Evaluation of Antioxidant Activity on Leaves of *Clausena excavata* Burm.f. (Pyin-Daw-Thein)

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Abstract

The aim of this study was to screen the phytochemical and antioxidant activity of water and ethanol extracts of leaves on *Clausena excavata* Burm.f. (Rutaceae). The plant is locally known as pyin-daw-thein in Myanmar and one of the natural sources of medicinal natural plant. In this study, *C.excavata* was screened to show the presence of chemical constituents in the leaves. The phytochemical investigation indicated that alkaloids, glycosides, carbohydrates, phenolic compounds, saponins, flavonoids, tannins, α-amino acids, reducing sugars, starch and organic acids were present in the leaves of *C.excavata*. The measurement of free radical scavenging activity (% RSA) of water and ethanol extracts of *C.excavata* leaves was performed to display potent antioxidant activity in order to reaction with 1,1-diphenyl- 2-picrylhydrazyl radical (DPPH) by using UV-double beam spectrophotometer at wavelength of 517 nm and ascorbic acid was used as a standard. *In vitro* radical scavenging activity via DPPH assay revealed that ascorbic acid (IC_{50}=1.23 µg mL^{-1}) and water and ethanol extract of *C.excavata* leaves were (IC_{50} = 63.76 µg mL^{-1}) and (IC_{50} = 76.23 µg mL^{-1}) respectively.

Keywords :Clausena excavata, DPPH, UV absorption, phytochemicals

1. INTRODUCTION

1.1 *Clausena excavata* Burm.f.

Clausena excavata is a medicinal plant widely distributed in Southeast Asia and is known by unique local names, such as Chamat in Thailand and Jia huang pi in China. In Malaysia the plant is locally known as “Cherek hitam” and “Kemantu hitam. In Tamil Kattukaruveppilai or Kattu veppilai” and In Thailand, it is known as “San Soak”(Khare, 2007).*Clausena* is a genus of about fourteen species of ever green tree, occurring mostly in India and tropical Asia (Shier,1983). The plant is easy to grow and, free of pests and diseasesleaves.*C.excavata* possesses strong antioxidantproperties and is a rich source of polyphenol compounds such as coumarins, carbazole alkaloid, and flavonoid glycosides.

1.2 Medicinal Properties of *C.excavata* Burn. f. Leaves

This plant isused traditionally in the treatment of abdominal pain, Snakebite and as a detoxification agent.A decoction of the roots is drunkfor bowel complaints, chiefly

colic.Decayed teeth can be treated using powdered rootstock, whereas its stem is given in colic with or without diarrhea. The expressed juice of the plant is used in Java for coughs, as vermifuge(Sharma, 1998).The leaves are used in folklore medicine for the treatment of several illnesses such as malaria, headache, abdominal pain, dysentery, pulmonary tuberculosis, diarrhoea, cold, wound, snake-bite, and poisoning. The plant also possessed immune-modulatory, analgesic, anti-inflammatory, antivirus, anticancer, antioxidant and antifungal activities.

1.3Botanical Description of *C.excavata* Burm. f. Leaves

C.excavata grows as a slender shrub 1–4 m in height. Leaves are compound with approximately 10–30 leaflets. Leaflets are ovate, 3-6 cm long, approximately 1.5cm wide, asymmetrical, finely hairy and aromatic with a distinctive aniseed or sarsaparilla smell. The plants can produce a compound inflorescence of pale green or cream coloured flowers in the leaf axils. Fruit are small, hairy, fleshy and are red at maturity. Trees and leaves of *C.excavata* were shown in Figure 1.1.

1.4Scientific Classification of *C.excavata*Burm.f.

Family	-	Rutaceae
Genus	-	*Clausena*
Species	-	*C. excavata*
Kingdom	-	Plantae
Myanmar name	-	Pyin- daw- thein
Botanical name	-	*Clausena excavata* Burm.f.

(a) (b)

Figure 1.1Photographs of *C.excavata* Burm. f. tree and leaves

1.5Antioxidant Activity and Free Radicals

Nowadays, there is an increased occurrence of various diseases like cardiovascular disease, neurological disorders, cancer, diabetes and autoimmune disease due to the presence of free radicals. Antioxidant is the agent that neutralizes the effect produced by free radical (Fang, 2002).These radicals damage various intracellular macromolecules to include DNA,protein, and lipids. Antioxidants have the ability to

prevent oxidative damage and inhibit inflammatory conditions by nullifying the activities of free radicals.

The addition of synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) has been used in the food industry to control lipid oxidation in foods. However, the use of these synthetic antioxidants has potential health risks and toxicity (Wong et al., 2006). Flavonoids, also called polyphenols, commonly occur as glycosides in plants (Pietta, 2000). It is important to search for antioxidants from natural sources to replace synthetic ones. Currently, the search for plant sources of antioxidants is gaining momentum with *C.excavata* (Rutaceae family) among the plants targeted.

2. MATERIALS AND METHODS

2.1 Sample Collection

The fresh mature leaves of *C.excavata* Burm. (pyin-daw-thein) were collected from the Inseinmarket, Insein Township, Yangon.

2.2Phytochemical Screening of *C.excavata* Leaves

Phytochemical screening of selected leaves was assessed to show the presence of various secondary metabolites in *C.excavata* leaves.

2.3Determination of Antioxidant Activity (DPPH) Assay of Water and Ethanol Extracts of *C.excavata* Leaves

2.3.1 Preparation of Solutions

(i) Preparation of 0.002% DPPH solution

DPPH (0.002g) was dissolved in ethanol and made the volume to 100mL. This solution was freshly prepared in the brown coloured flask.

(ii) Preparation of test sample solution

The stock solution (1000 µg mL^{-1}) of crude extracts of *C.excavata* leaves was prepared and diluted with ethanol to get the sample solution of 400,200,100, 50,25,12.5, 6.25, 3.125, 1.5625, 0.7813 and 0.3906 µgmL^{-1} concentrations.

(iii) Preparation of standard solution

The stock solution (100µgmL^{-1}) of standard ascorbic acid was prepared to get 6.25,3.125, 1.5625,0.7813 and 0.3906 µgmL^{-1} concentrations.

(iv) Preparation of blank solution

Blank solution was prepared by mixing 1.5mL of each sample solution with 1.5 mL of ethanol.

2.3.2Procedure for the DPPH Assay

Antioxidant activity determination was performed using DPPH method by usingSpectrophotometer (UV-Vis1800, Shimadzu) at Chemistry Department, West Yangon University according to the method of Burda and Oleszek (2001). Each extract (1.5 mL) was added to 1.5 mL of ethanolic DPPH solution until the colour of sample became purple. Then, the mixture was shaken using a vortex and left to stand at room temperature for 30 minutes in a dark place. The absorbance of the solution was measured at 517 nm.

3. RESULTS AND CONCLUSION

3.1 Phytochemical Screening of *C.excavata* Leaves

The phytochemical screening of selected plant, *C.excavata*leaves extracts was assessed and the results pertaining to the experiments are shown in Table 3.1.

3.2 Screening of Antioxidant Activity of Standard Ascorbic acid, Water and Ethanol Extracts of *C.excavata* Leaves

DPPH radical is scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H. The colour changes from purple to yellow colour after reduction which can be quantified by its decrease of absorbance at wavelength at 517 nm. The radical scavenging activity was expressed as in term of percent inhibition, (IC_{50}). The percent inhibition (IC_{50}) values in µg mL^{-1}were calculated by linear regression equation.

% RSA of standard ascorbic acid was indicated in Table 3.2, and Figure 3.2. % RSA of water extract was shown in Table 3.3 and Figure 3.3 and ethanol extract was shown in Table 3.4 and Figure 3.4 respectively.

Table3.1 Qualitative Phytochemicals Results of *C.excavata* Leaves

No.	Chemical Constituents	Extract	Test Reagents	Observation	Inference
1.	Alakaloids	1% HCl	(i) Dragendorff's (ii) Wagner's (iii) Mayer's	Brown ppt. Brown ppt. White ppt.	+ + +
2.	Glycosides	Distilled water	10% Lead acetate	White ppt.	+
3.	Carbohyrates	Distilled water	Molisch's	Red ring	+
4.	Phenolic compounds	Distilled water	5 % ferric chloride	Brown ppt.	+
5.	Saponins	Distilled water	Distilled water	Frothing	+
6.	Flavonoids	EtOH	Shinoda's	Orange solution	+
7.	Tannins	Distilled water	Ferrous sulphate	Green ppt.	+
8.	α-amino acids	Distilled water	Ninhydrin	Purple colour	+
9.	Reducing sugars	5M H_2SO_4	Benedict's solution	Brown ppt.	+
10.	Starch	Distilled water	Iodine	Red colour	+
11.	Organic acid	Distilled water	Bromocresol green	Blue colour	+
	(+) presence	(-) Absence			

Table 3.2 DPPH Radical Scavenging Activity (% RSA) of Ascorbic Acid

Concentration (µg mL^{-1})	% RSA (Ascorbic acid)	IC_{50} (µg mL^{-1})
0.7813	45.89	
1.5625	53.07	
3.125	79.23	1.23
6.25	81.28	
12.5	86.87	

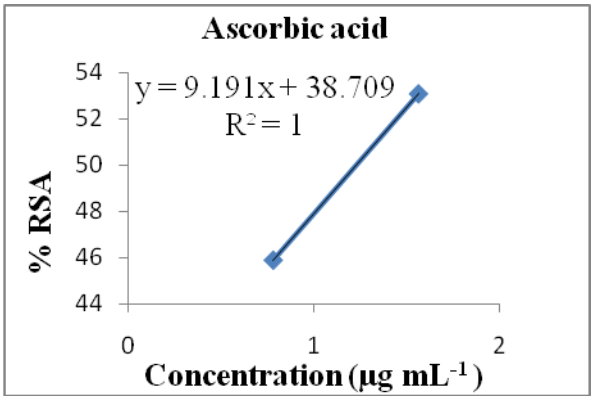

Figure 3.2 Antioxidant Activity of Standard Ascorbic Acid

Table 3.3 DPPH Radical Scavenging Activity (%RSA) of Water Extract

Concentration (µg mL^{-1})	% RSA	IC_{50} (µg mL^{-1})
12.5	23.58	
25	36.92	
50	45.13	63.76
100	62.82	
200	83.33	

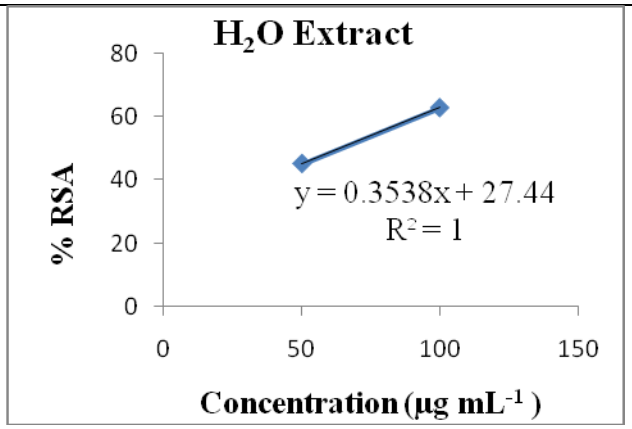

Figure 3.3 Antioxidant Activity of Water Extract of *C.excavata* Leaves

Table 3.4 DPPH Radical Scavenging Activity (% RSA) of Ethanol Extract

Concentration (µg mL^{-1})	% RSA (EtOH Extract)	IC_{50} (µg mL^{-1})
25	37.69	
50	44.35	76.23
100	55.12	
200	79.23	
400	80.00	

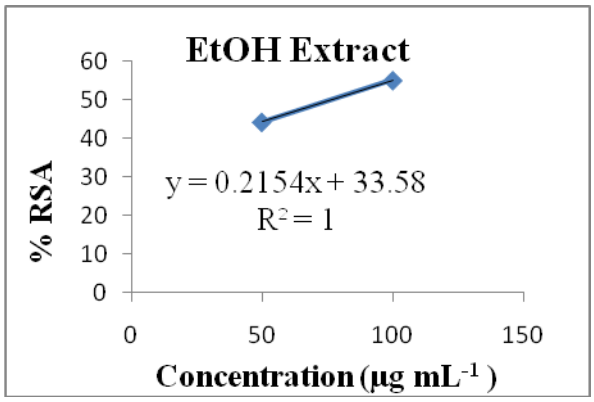

Figure 3.4Antioxidant Activity of Ethanol Extract of *C.excavata* Leaves.

4. CONCLUSION

The present study of *C.excavata* leaves revealed the following inferences. The observations with regards to the phytochemical investigation indicated that alkaloids, glycosides, carbohydrates, phenolic compounds, saponins, flavonoids, tannins, α-amino acids, reducing sugars, starch and organic acids were present in the leaves of *C.excavata*. From the phytochemical results, it was found that *C.excavata*was shown antioxidant activity due to the presence of phytoconstituents of phenolic and flavonoid compounds in the leaves sample. From the experimental results of *in vitro* radical scavenging activity via DPPH assay, it was revealed that ascorbic acid (IC_{50} = 1.23 µg mL^{-1}) and water and ethanol extract of *C.excavata* leaves were (IC_{50} = 63.76 µg mL^{-1}), and (IC50 = 76.23 µg mL^{-1}) respectively. In the determination of antioxidant activity, the smaller is the IC_{50} value and the greater the antioxidant activity. Therefore, the water extract was indicated more potent radical scavenging activity than ethanol extract and it was clearly showed that *C.excavata*can be used as a potent source of natural antioxidant.

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