Asia-Pacific Journal of Science and Technology: Volume: 27. Issue: 06. Article ID.: APST-27-06-13. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Technology Transfer Affairs Division,
Khon Kaen University, Thailand

Phytochemical screening, total phenolic, flavonoid and alkaloid contents, cytotoxicity, and antioxidant activities of *Capparis monantha*, Jacobs extracts

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Received 13 May 2021
Revised 12 October 2021
Accepted 30 December 2021

Abstract

Capparis monantha Jacobs is a medicinal plant that is found on the coast of Thailand. These herbal medicines are used by the local people to cure hemorrhoids and as an anticancer treatment. The goal of the current examination was to analyze the phytochemical constituents, total phenolic, total flavonoid, total alkaloid contents, antioxidant activities, and anticancer properties against three cancer cell lines (KB cell line, MCF-7 cell line, and small NCI-H187 cell line) in the stem of *C. monantha* Jacobs. The *C. monantha* Jacob's powder was extracted with hexane, dichloromethane, acetone, and methanol, by using a maceration method. The increasing polarity of the organic solvent was used to fractionate the extracted residue. The qualitative phytochemical screening reveals all extracts containing tannin and phenolic, flavonoid, triterpenoid, and alkaloid. The total phenolic was carried out using the Folin-Ciocalteau method. The highest total phenolic, total flavonoid, and total alkaloid contents were found in acetone extract (447.3 mg GAE (Gallic Acid Equivalent)/g extract), hexane extract (579.2 mg QE (Quercetin Equivalents)/g extract), and methanol extracts (13.80 mg AE (Aescin Equivalents)/g extract), respectively. The antioxidant activity is high in acetone extract. Cytotoxicity measured by Resazurin microplate assay of all extract fraction at 50 mg/mL against three cancer cell lines (KB cell line, MCF-7 cell line, and NCI-H187 cell line) were found to less than 50% inhibited cancer growth. According to the literature, it is most likely that this is the first report on phytochemical screening, total phenolic, flavonoid, and alkaloid contents, cytotoxicity, and antioxidant activities of *C. monantha* Jacobs.

Keywords: Phytochemical, Antioxidant activity, Cytotoxicity, Phenolic, Flavonoid, Alkaloid, *Capparis monantha* Jacobs

1. Introduction

Free radical, unstable molecular compounds generally look for electrons in biomolecules that cause damage to various cells in the body. They can lead to various degenerative diseases such as osteoarthritis, Alzheimer's disease, diabetes, and Parkinson's, etc. Antioxidants, also called free-radical scavengers, are compounds that can delay or inhibit the cell damage caused by free radicals [1]. The natural antioxidant from the various plants has some advantages as the samples can be gotten effortlessly, economically, and basically with no side effects [2]. Natural antioxidants occur in the form of food and plants. There are rich secondary metabolites such as triterpenoids, flavonoids, tannin, alkaloids, saponins, and glycosides [3]. *Capparis monantha* Jacobs (*Capparaceae*) is commonly known as 'Nammona', that found in Prongsalotnative shrub, Phetchaburi province, Thailand.

The genus Capparis has been utilized in traditional Thai medicine to alleviate ailment in the pharynx, fever, treatment for hemorrhoids, and cancer [4]. Through literature research, it has been found that there is no previous scientific report on its pharmacognostical and phytochemical properties. The information accessible mostly concentrates on its traditional use by local people. In the present study, the stem of *C. monantha* Jacobs was examined for its phytochemical constituents, the resolution of the total phenolic, flavonoid, and alkaloid content,

and antioxidant scavenging activity by spectrophotometric methods. This research aimed to analyze the qualitative primary phytochemical screening, the quantity of total phenolic, flavonoid, and alkaloid contents of the hexane, dichloromethane, acetone, and methanol extracts of *C. monantha* Jacobs. Also, to determine the antioxidant activity and cytotoxicity of *C. monantha* Jacobs extracts.

2. Materials and methods

2.1 Plant materials

The stem of *C. monantha* Jacobs was randomly sampled from Prongsalot, Banlat, Phetchaburi province, Thailand, in December 2018. The sample was certified by botanists at Faculty of Agricultural Technology, Phetchaburi Rajabhat University, Phetchaburi, Thailand.

2.2 Preparation of plant extracts

The fresh stem was cleaned under running water and dried in the sun. The stem was ground to a coarse powder using the WF-10 grinder (Thai Grinder, Thailand). The powdered sample (300 g) was extracted using the maceration method, which involved increasing the polarity of the solvent with hexane, dichloromethane, acetone, and methanol, respectively. The obtained fraction was filtered by filter paper Whatman No. 1. The solvent was removed from each filtrate using a rotary evaporator. Finally, the crude extracts were stored in brown vials maintained at 4 °C for further use.

2.3 Analytical methods

2.3.1 Phytochemical screening

The crude extracts were investigated for the presence of alkaloids, tannins, phenolic, flavonoids, saponins, triterpenoids, and cardiac glycoside. All experiments were done in triplicate using the modified method of Harborne [5]. The alkaloids were analyzed by mixing 10 mg of extract with 1 mL of 2 N hydrochloric acid solution (HCl) and 9 mL of distilled water (dH_2O). Then the prepared solution was heated to boiling for 2 min and filtered. The cleared solution was mixed with 2 mL of Dragendroff's reagent. The presence of alkaloids will cause the mixture to turn orange or orange/red in color. The tannins and phenolics were determined by combining 10 mg of sample with 20 mL of dH_2O. The mixture solution was heated for 5 min and filtered. The filtrate (5 mL) was further mixed with 2-3 drops of Iron (III) chloride ($FeCl_3$). The presence of tannins and phenolic will cause the mixture solution to be brownish-green or dark blue color. Flavonoids were determined by adding 10 mL of dH_2O to 10 mg of sample, heating to boiling for 5 min, and filtering. To the mixed solution, add Magnesium metal powder was added to the mixture solution 1 mL concentrate HCl, and 1 mL Pentyl alcohol. The resultant blue-green color of the amyl alcohol layer confirmed the presence of flavonoids. The saponins were measured using a 10 mg sample mixed with 10 mL dH_2O, heated to boiling, cooled, then shaken for 1 min. The presence of saponins was indicated by the formation of a stable foam (10 min). The triterpenoids were examined by adding 5 mL of chloroform to 10 mg of the crude extract. The prepared solution was added with acetic anhydride and concentrate sulfuric acid (conc. H_2SO_4) (2:1, (v/v)). The presence of triterpenoids produces a reddish-purple, whereas the presence of steroids produces a blue-green in color. The basic structure of cardiac glycoside contains deoxy sugar and cardenolides, which were tested for deoxy sugar using the Killer Killiani test. The 10 mg sample was dissolved in a solution of 1% iron (III) sulfate ($Fe_2(SO_4)_3$) in 5% glacial acetic acid (CH_3COOH), then 1-2 drops of conc.H_2SO_4 were added. A blue color indicates the presence of deoxy sugar. For cardenolides analysis, the Kedde test is used. In 5 mL of ethyl acetate, 10 mL of extract was dissolved. The mixture was then added with 1-2 drops of Kedde's reagent (2 % of 3,5-dinitrobenzoic acid in menthol and 7.5 percent potassium hydroxide (KOH), 1:1, (v/v). The presence of cardenolides causes the color to be blue or violet.

2.3.2 Determination of total phenolic content

The Folin–Ciocalteau method [6] was used to determine total phenolic content. The samples (1 mg/mL of extract in ethanolic solution) were mixed with 1.25 mL of 10% Folin–Ciocalteu reagent, incubated for 3 min at room temperature (RT), then added 2 mL of 7.5 percent (w/v) sodium carbonate (Na_2CO_3) and allowed to develop for 30 min at RT. The absorbance at 765 nm of resulting blue color was determined using a spectrophotometer (Shimadzu UV-1240, Japan). Total phenolic content was calculated using a gallic acid standardization curve; total phenolic content is expressed as mg gallic acid equivalent per gram of extract (mg GAE (Gallic Acid Equivalent)/ g extract).

2.3.3 Determination of total flavonoid content

The total flavonoid concentration was determined using an aluminum chloride colorimetric assay [7]. 500 µl of a sample (1 mg/mL of extract in ethanol solution) was mixed with 150 µl of 5 % (w/w) of sodium nitrite ($NaNO_2$) solution. The reaction mixture was left at RT for 6 min. The solution of 10 % $AlCl_3$ (150 µl) was further added to the mixture, re-incubated at RT for 6 min. Then 2 mL of 4 % (w/v) NaOH solution was added, bringing the total volume to 5 mL using dH_2O. The mixed solution was left at RT for 15 min. The absorbance was measured at 510 nm by a spectrophotometer. The total flavonoid content was calculated from a standardization curve of quercetin; total flavonoid content represented as mg quercetin equivalent per g extract (mg QE (Quercetin Equiv alents)/g extract).

2.3.4 Determination of total alkaloid content

The 1 mg extract was mixed with 1 mL 2 N HCl, then filtered through filter paper. The prepared solution was transferred to a separatory funnel, then washed three times with 10 mL of chloroform. Then, to produce a complex, 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added and shaken to reaction. The solution was then extracted with chloroform (1, 2, 3, and 4 mL, respectively) by a vigorous shaking. The extracts were obtained, chloroform was used to make-up the final volume to 10 mL. The absorbance at 470 nm was measured by using spectrophotometer; the total alkaloid content is expressed as mg atropine equivalent per g extract (mg AE (Aescin Equivalents)/ g extract) [8].

2.3.5 Cytotoxic activity

Resazurin microplate assay (REMA) was used to investigate the cytotoxic activity of the extracts from *C. monantha* Jacobs against the KB cell line (epidermoid carcinoma of the oral cavity), MCF-7 cell line (breast adenocarcinoma), and NCI-H187 cell line (small cell lung carcinoma) [9]. 45 µl of each cell line (concentration of MCF-7 cell line was 7 x 10^4 cells/mL and KB cell line and NCI-H187 cell line were 9 x10^4 cells/mL) was mixed with 5 µl of 5% dimethylsulfoxide (DMSO) in 384-well plate and incubated at 37 °C for 5 days in 5% CO_2 incubator. The plate was then re-incubated for 4 hours with 12.5 µl of 62.5 ppm resazurin reagent added to each well. The SpectraMax M5 multi-detection microplate reader was used to measure the fluorescence emission spectrum (Molecular Devices, USA). Tamoxifen, doxorubicin, and ellipticine were utilized as positive controls in the experiment, whereas 5% DMSO was employed as a negative control. The % inhibition was carried out by the equation: % inhibition = [1-(Fs / Fc] x 100, where Fs is the average fluorescence emission of sample and Fc is the average fluorescence emission of control.

2.3.6 Antioxidant Activity Assay

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay was used to investigate antioxidation activity [10]. 3 mL of the sample was treated with 3 mL of ethanolic solution containing 1 mg/mL DPPH. For 30 min, the mixed solution was kept at RT in a dark environment. The absorbance was then measured at 515 nm using spectrophotometer. Gallic acid was used to be a positive control. The equation $[(A_{DPPH} - A_{sample})/ A_{DPPH}] \times 100$ % was used to calculate the DPPH radical scavenging capability, whereas A_{DPPH} is the absorbance of the DPPH ethanolic solution and A_{sample} is the absorbance of the sample. The antioxidant activity was expressed as IC_{50}, the sample concentration required to inhibit 50 % of DPPH radicals.

3. Results and discussion

3.1 Plant extracts

Table 1 summarized the extraction yield of each tested plant, which prepared by maceration method at room temperature using hexane, dichloromethane, acetone, and methanol, respectively. The extraction yields of each extract were 0.59±0.01, 0.64±0.01, 1.82±0.01 and 4.55±0.08% (w/w), respectively (Table 1).

Table 1 Physical properties and quantities of the extracts of *C. monantha* Jacobs.

Extract	Properties / color/ weight (%yield)
Hexane	viscous liquid / dark yellow / 1.78 (0.59 ± 0.01)
Dichloromethane	viscous liquid / dark brown/ 1.94 (0.64 ± 0.01)
Acetone	viscous liquid / dark red and brown / 5.46 (1.82 ± 0.01)
Methanol	viscous liquid / dark red and brown /13.65 (4.55 ± 0.08)

3.2 Phytochemical screening

The phytochemical analysis shows that all extract fractions comprise common classes of phytochemicals such as phenolic, flavonoids, alkaloids, and triterpenoids (Table 2). Saponins, steroids, and cardiac glycoside were not detected in all extracts. The phytochemical screening is used to determine the secondary metabolite and to develop new drugs. The phytochemicals protect the biotic and abiotic environment. The differences in extraction solvents play an important role in the extraction of the secondary metabolites in these kind of plants [11-13]. Tannins and natural polyphenols play an important biological role and have been proposed for antitumor, anti-plasmin inhibitors, and antioxidants [14-15]. Tannins are commonly found in various parts of plants and are key to regulating the growth of tissue and to protect plants from insect [15-16]. Triterpenoids are biosynthesized from squalene. These compounds are classified as tetracyclic triterpenoids and pentacyclic triterpenoids. Triterpenoids play essential biological roles and have been proposed for antioxidants, anti-glycation, and anti-diabetes [17-18]. Flavonoids, polyphenol compounds, play essential biological roles have been proposed for antioxidant, anticancer, and anti-microbial [19-21]. Alkaloids, alkaline compounds are present in several plants. They play essential biological roles and have been proposed for anti-inflammatory, anti-diabetic, antioxidant, and anti-bacterial [22-24].

Table 2 Phytochemical compound of *C. monantha* Jacobs.

Phytochemical	Test	Extracts			
		Hexane	Dichloromethane	Acetone	Methanol
Phenolics and tannins	Ferric chloride	+	+	+	+
Saponins	Foam test	-	-	-	-
Triterpenoids	Liebermann-Burchard	+	-	+	+
Steroids	Liebermann-Burchard	-	-	-	-
Flavonoids	Cyanidin	+	+	+	+
Cardiac glycosides	Kedde's reagent	+	+	+	+
	Killer-kiliani	-	-	-	-
Alkaloids	Dragendroff's reagent	-	+	+	+

Note: (+): presence, (-): absence of phytochemical.

3.3 Total phenolic content

The total phenolic content of the hexane, dichloromethane, acetone, and methanol extracts, determined by standardization curve of gallic acid; $y = 8.2117x + 0.0675$, $R^2 = 0.9909$, were 39.8, 163.5, 447.3 and 223.5 mg GAE/g extract, respectively (Table 3). In the present study, the acetone extract was shown to be the highest of the total phenol contents, and hexane extract was shown to be lowest. These results suggested that the phenolic content extracted from *C. monantha* Jacobs was affected by the solvent polarity. The scientific report conducted by Teh *et al.* (2014), hexane solvent has a high ability to extract hydrophobic compounds such as lipids and steroids, but it has a low ability to extract hydrophilic compounds such as phenolic content [13]. Accordingly, the extraction of total phenol content relies on the polarity of organic solvents.

3.4 Total flavonoid content

Total flavonoid contents were determined by the aluminium chloride colorimetric assay. The linear equation of quercetin; $y = 0.0005x–0.0038$, $R^2=0.9936$, was used to calculate the total flavonoid contents. The total flavonoid contents of *C. monantha* Jacobs are shown in Table 3. These results revealed that the hexane extract contained the highest (579.2 mg QE/ g extract) amount of flavonoid contents, whereas methanol extract contained the lowest amount of flavonoid contents (63.2 mg QE/ g extract). This result is an approach to *Rajhi et al.* (2019), who mentioned that the total flavonoid content of methanolic extracted of *Capparis spinosa* L. leaves was 39.6 mg QE/g extract [25]. The flavonoid, a secondary metabolite, consists of a large free OH group related to the potency of antioxidant activity [26].

3.5 Total alkaloid content

The total alkaloid content was investigated by the bromocresol green complex method. The total alkaloid contents were calculated from the linear equation; $y = 1.7957x +0.0009$, $R^2=0.9994$. The results of the total alkaloid contents of *C. monantha* Jacobs extracts is given in Table 3. The total alkaloid contents varied from 2.0-13.8 mg AE/g extract. Methanol extract is rich in alkaloids. Earlier scientific reports described how the isolation of alkaloids in fruits of *C. Spinosa* were shown to have compounds with many health benefits such as Cappariloside A, stachydrin, hypoxanthine, and uracil [27].

Table 3 The total phenolic, total flavonoid, total alkaloid contents, and antioxidant activity of four extracts of *C. monantha* Jacobs.

Extracts	Antioxidant Activity ($IC_{50,}$ mg/mL)	Total phenolic contents (mg GAE/g extract)	Total flavonoid contents (mg QE/g extract)	Total alkaloid contents (mg AE/g extract)
Hexane	-*	39.83 ± 0.15	579.00 ± 0.20	2.07 ± 0.02
Dichloromethane	0.1663 ± 0.0002	163.33 ± 0.15	547.23 ± 0.25	7.46 ± 0.01
Acetone	0.0740 ± 0.0010	447.27 ± 0.25	539.23 ± 0.25	8.43 ± 0.02
Methanol	0.4541 ± 0.0004	223.40 ± 0.36	63.20 ± 0.20	13.80 ± 0.01

*mean unable to determine IC_{50}.

3.6 Cytotoxic activity

Cytotoxicity is useful to find out the toxicity of the substance. This study was to implement the cytotoxic activities of four extracts of *C. monantha* Jacobs against the KB cell line, MCF-7 cell line, and NCI-H187 cell line by REMA assay. The result of different extracts fraction is shown in Table 4. The results of the REMA assay of *C. monantha* Jacobs shows less effect on 3 cell lines, with the percentage of growth inhibition less than 50. The results indicated that *C. monantha* Jacobs could not be used to control cancer or for cancer treatment. In this study, the *C. monantha* Jacobs extracts revealed the presence of phenolic flavonoids, alkaloids, and triterpenoids (Table 2) and showed high levels of total phenolic and total flavonoid contents (Table 3). Additionally, not all the phenolic and flavonoids compounds showed potential growth inhibition on various kinds of cancer cells [20], indicating that the *C. monantha* Jacobs extracts do not contain the active compounds against the cancer cells. In summary, the *C. monantha* Jacobs extracts have great significance for their traditional use in the treatment of various disorders other than cancer.

Table 4 Cytotoxicity Activity of *C. monantha* Jacobs extracts against KB cell line, MCF-7 cell line, and NCI-H187 cell line at concentration 50 µg/mL.

Extracts	KB cell	MCF-7 cell	NCI-H187
Hexane	Inactive	Inactive	Inactive
Dichloromethane	Inactive	Inactive	Inactive
Acetone	Inactive	Inactive	Inactive
Methanol	Inactive	Inactive	Inactive

-inactive mean the growth inhibition of cancer cell less than 50 %

3.7 Antioxidant activity

Antioxidants, free radical scavengers, produce the duration of the oxidation process. The DPPH assay determined antioxidants. The DPPH radical (purple color) showed accepted electron radicals from antioxidants to form the reduced DPPH (yellow color). The change of color from purple to yellow was measured at absorbance of 517 by spectrophotometer. The degree of discoloration reveals that the ability of antioxidants in the sample to donate hydrogen or electrons [28]. Moreover, the DPPH assay is normally used for testing in-vitro antioxidant activity. The half-maximal inhibitory concentration (IC_{50}) of four extracts were determined and compared with gallic acid, as shown in Table 3. The hexane extract was unable to determine IC_{50} value, whereas dichloromethane, acetone and methanol extracts showed IC_{50} values of 0.1664, 0.0750 and 0.4541 mg/mL, the linear equation of each extract fraction were y=264.8x+5.9433 (R^2=0.9734), y=527.37x+10.64 (R^2=0.9996), y=73.65x+15.83 (R^2=0.9721), respectively. The standardization curves of gallic acid were carried out by the following equation: y=22.928x+1.626, R^2=0.994, and expressed the IC_{50} values of 0.0021 mg/mL. These results revealed the highest antioxidant activity as acetone extract, followed by dichloromethane and methanol extracts. Although gallic acid had higher antioxidant activity than the four other extracts, the extracts still showed good antioxidant activity. The *C. monantha* Jacobs stem's consumption can be useful in inhibiting oxidative stress-related degenerative, acute, or chronic diseases [11].

3.8 Correlation of antioxidant activity, total phenolic, flavonoid, and alkaloid contents

One-way ANOVA tested the correlation of antioxidant activity, total phenolic, flavonoid, and alkaloid contents in SPSS software version 11.5 (SPSS, Chicago, IL, USA). Statistical significance was defined as $p< 0.05$ and $p< 0.01$. The result is shown in Table 5. The results show that high content of total phenolic relates to high antioxidant activity, statistically significant as $p< 0.01$, but low of flavonoid compounds, statistically significant as $p< 0.05$. This occurs due to the phenolic compound's generally are high antioxidant activity compared to flavonoids. It occurs due to preliminary phytochemical screening of each extract. The results confirmed the

presence of common classes of secondary metabolites in the *C. monantha* Jacobs stem extract, including phenol and flavonoid compounds. The plant extract containing a high amount of phenol has antioxidant activity that is higher than other extracts [29-30].

Table 5 Correlation coefficient between antioxidant Activity, total phenolic, flavonoid, and alkaloid contents.

Correlation	Total phenolic contents (mg GAE/g extract)	Total flavonoid contents (mg QE/g extract)	Total alkaloid contents (mg AE/g extract)	Antioxidant Activity (IC_{50}, mg/mL)
Total phenolic contents (mg GAE/g extract)	1	-0.989*	0.740	-0.991**
Total flavonoid contents (mg QE/g extract)	-0.989*	1	-0.707	0.966*
Total alkaloid contents (mg AE/g extract)	0.740	-0.707	1	-0.686
Antioxidant Activity (IC_{50}, mg/mL)	-0.991**	0.966*	-0.686	1

*significant at 0.05
**significant at 0.01

4. Conclusion

In this study, the *C. monantha* Jacob's stem extracted by different solvents **(**hexane, dichloromethane, acetone, and methanol**)** revealed the presence of tannins, phenolic, flavonoids, triterpenoids, and alkaloids**.** The quantitative phytochemical, total phenolic compound, total flavonoids, total alkaloids, antioxidants, and cytotoxicity in the stem of *C. monantha* Jacobs were detected**.** The antioxidant capacity test showed that the highest gallic acid equivalent and total alkaloid is achieved by methanol extract**.** These results suggest that *C. monantha* Jacobs stem is a potential source of antioxidants and could be used as a natural antioxidant**.** Also, the methanol extracts showed the highest total alkaloid content**.** This study provides only necessary information about *C. monantha* Jacobs, and further studies are required to isolate, identify, and determine the compounds.

5. Acknowledgements

This research was financially supported by the Plant Genetic Conservation Project, the division of chemistry, faculty of science and technology, and Phetchaburi Rajabhat University**.** We would like to thank the national center for genetic engineering and biotechnology **(**BIOTEC**)** for advice on cytotoxicity experiments**.**

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