Phytochemical and Antioxidant Activity of *Acacia concinna* (Willd.) DC.Fruits

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Abstract

Medicinal plants contain numerous biologically active compounds which are help improve human life. *Acacia concinna* (Willd.) DC. belongs to the family Mimosaceae under the order Fabales. It was collected from North Okkalapa Township, Yangon Region. The plant was widely distributed in Myanmar, Southern China and Malaysia. The plant was a climbing shrub with thorny branches having brown smooth stripes. The preliminary phytochemical investigation of *Acacia concinna* (Willd.) DC. fruits revealed the presence of alkaloids, glycoside, reducing sugars, α-aminoacids, phenolic compounds, saponins, carbohydrates, steroids, tannins, flavonoids and starch. In this study, the aqueous extracts of*Acacia concinna* (Willd.) DC. fruits showed better antioxidant activity than the ethanolic extracts by using DPPH radical scavenging assay.
Keywords: Antioxidant activity, DPPH

Introduction

Medicinal plants have been used by mankind since time immemorial. Plants can synthesize a wide variety of chemical compounds that perform important biological functions. The genus *Acacia* belongs to the family Mimosaceae. In Myanmar, the fruits are also called Kin-Mon-Thee. There are some 1350 species of *Acacia* found throughout the world.The name *Acacia* is derived from the Greek word 'akis' meaning 'sharp point'. *Acacia concinna* (Willd.) DC. is a medicinal plant that grows in tropical rain forests of Southern Asia and the fruits of this plant are used for washing hair, for promoting hair growth. Although the pods of this plant are known to contain several saponins based on acacic acid. Its dried pods are powdered to produce shikakai powder. Shikakai is also used in traditional medicine to treat jaundice,constipation, skin problem, itching, pimples, hyperpigmentation, leprosy, psoriasis, gum infection and dandruff. The leaves, bark and pods have been used as herbal medicine for emetic, purgative and expectorant treatments.In Myanmar, India and Thailand , leaves used to prevent diabetes and skin diseases. The decoction of pods used for washing wounds and facilitating wound healing. The aim of the present research work is to examine the medicinal plant scientifically which have effective medicinal values. The objectives are to study the morphological characters of *Acacia concinna* (Willd.) DC. and to determine the preliminary phytochemical investigation and antioxidant activity of aqueous and ethanolic extracts of *Acacia concinna* (Willd.) DC. fruits by using DPPH radical scavenging assay.

Materials And Methods

The specimens used in this research were collected from North Okkalapa Township. The fruits were dried in shade for several days when completely dried, these were pulverized by grinding machine to get the powder and stored in an airtight container for the chemical study.The morphological and phytochemical tests were conducted at the Department of Botany, Dagon University. The preliminary phytochemical investigation of *Acacia concinna* (Willd.) DC. fruits were determined by the methods of Central for research in Unani Medicine (1987) and Trease and

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Evans (1987). The antioxidant activity, total phenolic content and total falvonoid content were carried out at the Department of Oriental Herb Science,Chonbuk University, Iksan in Korea.

Extraction of *Acacia concinna*(Willd.) DC. fruits

Each powder sample 100 g of *Acacia concinna* (Willd.) DC. fruits were extracted using different solvents including 99% of ethanol and distilled water. The samples were soaked in ethanol for 12 hours and distilled water that was boiled in water bath (60˚C) for one hour. The two extracts were filtered through a sheet of filterpaper (Whatman No. 1) and the filtrates were re-filtered through a 0.45 µm nylon membrane filter (GE Healthcare UK).The collected filtrated were dried in different processes. The ethanolic extract was concentrated using a rotary evaporator with water bath at (60˚C) and the aqueous extract was concentrated using a rotary evaporator with water bath at (80˚-90˚C). And then, the two extracts were dried by freeze drier at (-62˚ C).

Test for Antioxidant Activity

Preparation of DPPH (1,1-diphenyl- 2-picrylhydrazyl)

DPPH stock solution,0.002 g of DPPH in 50 ml of methanol. The solution was freshly prepared and stored in falcon tube wrapped with silver foil.

Preparation of Test sample solution

0.2g of test sample and 2 ml of methanol were thoroughly mixed by vortex mixer.Then, the mixture solutions were placed in centrifuge. After 10 minutes, the stock solutions were obtained.

Measurement of DPPH Radical Scavenging Activity by Spectrophotometeric method

The control solution was prepared by mixing 200 µl of methanol and 1.8 µl of DPPH. Similarly, the blank solution was prepared 2 µl of methanol only. The sample solution was prepared by mixing 40 µl of test sample solution, 160 µl of methanol and 1.8 µl of DPPH solution. All solutions were kept in the dark for 30 minutes. Then the absorbance of the solution was measured at 517 nm using a UV-1601 Shimadzu Spectrophotometer. Methanol was used as standard and control. These were done in triplicate. The percentage inhibition was calculated by using the following equation:

$$\%\text{inhibition} = (1 - S/C) \times 100$$

S=Absorbance ofsample , C= Absorbance of control

The results were shown in table (2).

Total phenolic content

Total phenolic content of each extract was determined by Folin-Ciocalteu's reagent. The sample 100 µl was prepared by mixing 2 ml of 2% Na_2CO_3. The mixture was left at room temperature for 3 minutes. Then, 100 µl of 50% Folin-Ciocalteu's reagent was added to the mixture and left for 30 minutes. The absorbance of the solution was determined at 700 nm using a UV-1601 Shimadzu Spectrophotometer. These were done in triplicate. The equivalent values of the extract were calculated by using the following equation:

Sample (Abs)-0.1523/0.8965

The results were shown in Table (2).

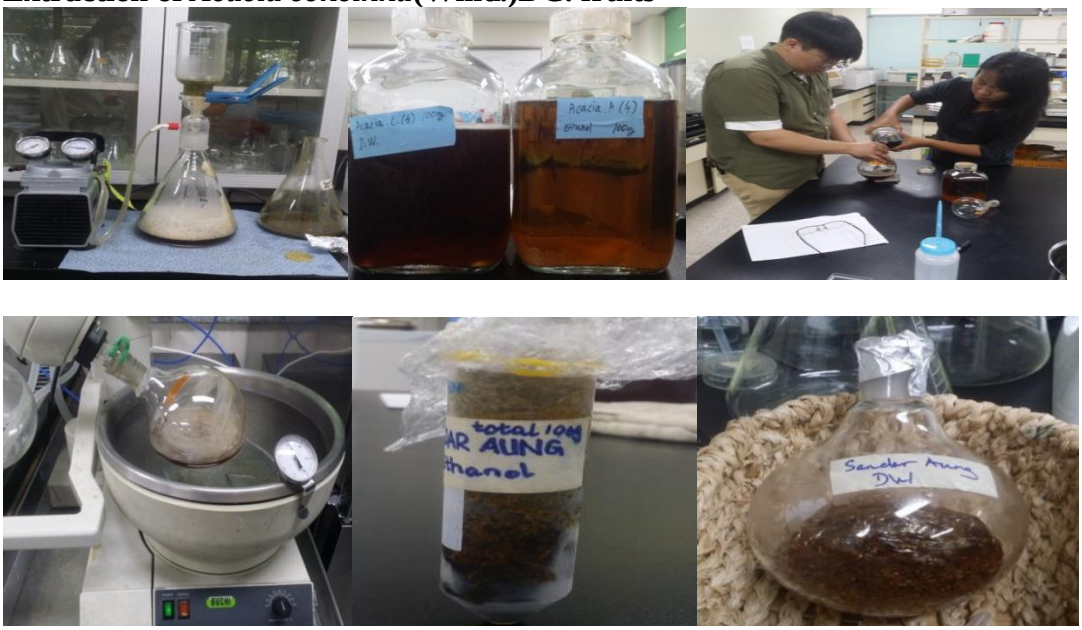
Total Flavonoid Content

Total Flavonoid content of each extract was determined by 10% $AlCl_3$.6 H_2O. The sample 250 µl, 1ml of distilled water and 75 µl of 5% $NaNO_2$ were mixed and incubated for 5 mins. Then, 150 µl of 10% $AlCl_3$.6 H_2O was added into the mixture. After 6 minutes of incubation, 500 µl of 1M NaOHwas added into the mixture and left for 11 minutes.The absorbance of the solution was determined at 500 nm using a UV-1601 Shimadzu Spectrophotometer. These were done in triplicate. The equivalent values of the extract were calculated by using the following equation:

Sample (Abs)-0.0848 / 0.0002

The results were shown in Table (2).

Extraction of *Acacia concinna*(Willd.)DC. fruits



RESULTS

Morphological Characters of *Acacia concinna* (Willd.) DC. Fruit

Scientific name -*Acacia concinna* (Willd.) DC.
Myanmar name - Kin-mun-gyin
English name - Soap acacia
Family - Mimosaceae

Prickly climbing shrub, red-brown at the tender shoots. Leaves alternate, bipinnate, paripinnate compound, petiolate, pulvinate and stipulate. Inflorescences 2-3 stalked globose head, Flowers bracteolate, bisexual, regular, actinomorphic, pentamerous. Calyx synsepalous, 5 - toothed. Corolla synpetalous, campanulate.Stamen numerous, filaments filiform, anther dithecous, basifixed, longitudinal dehiscence.Ovary long, marginal placentation. Pod oblong, slightly compressed, constricted between the seed.

Habit Leaves Inflorescence

Fruits Seeds

Preliminary phytochemical investigation of *Acacia concinna* (Willd.) DC. fruits

The preliminary phytochemical investigation of *Acacia concinna* (Willd.) DC. fruits revealed the presence of alkaloids, glycoside, reducing sugars, α- amino acids, phenolic compounds, saponins, carbohydrates, steroids, tannins, flavonoids and starch. The results were shown in table (1).

Table (1). Preliminary Phytochemical tests of *Acacia concinna* (Willd.) DC.fruits

Sr. No.	Test	Extract	Test Reagent	Observation	Results
1.	Alkaloids	1% HCL	(1) Wagner's reagent (2) Dragendroff's reagent	Cream ppt. Organe ppt.	+ +
2.	α-amino acids	H_2O	Ninhydrin reagent	Deep pink	+
3.	Glycosides	H_2O	10% lead acetate	White ppt	+
4.	Carbohydrates	H_2O	10% α-napthol + conc : H_2SO_4	Red ring	+
5.	Reducing sugars	H_2O	Benedict's solution	Yellow ppt.	+
6.	Starch	H_2O	Iodine solution	Blue black	+
7.	Saponins	H_2O	Distilled waters	Forthing	+
8.	Phenolic compounds	H_2O	Ferric chloride solution	Deep blue	+
9.	Flavonoids	95% ethanol	Conc : HCL and Mg burning	Pink	+
10.	Steroids	Petroleum ether	Acetic anhydride+ Conc : H_2SO_4	Pink	+
11.	Tannins	H_2O	1% ferric chloride solution	Brown	+

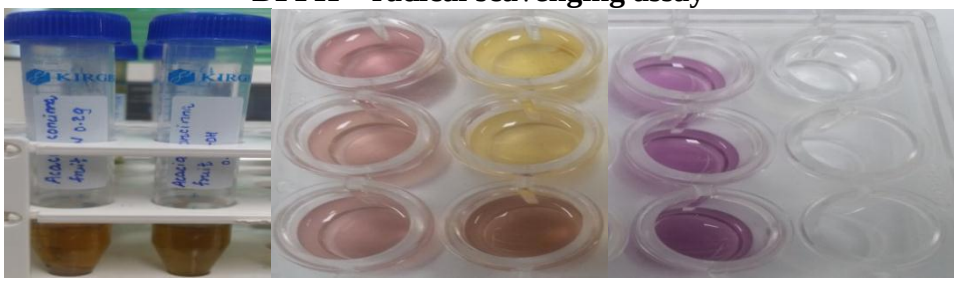
Absent=(-) Present =(+) ppt=precipitate

Antioxidant activity of *Acacia concinna*(Willd.) DC. Fruits

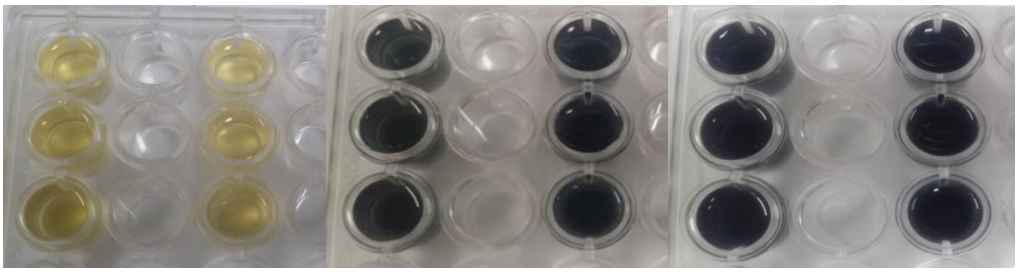
DPPH – radical scavenging assay

99% of ethanolic and aqueous extracts were used for screening of radical scavenging activity by DPPH method. Absorbance decreases as a result of a colour change from purple to yellow as the radical was scavenged by antioxidant through donation of hydrogen to form the stable DPPH-H. The data showed that DPPH solution was bleached with all the sample tested. The results were shown in Table (2).

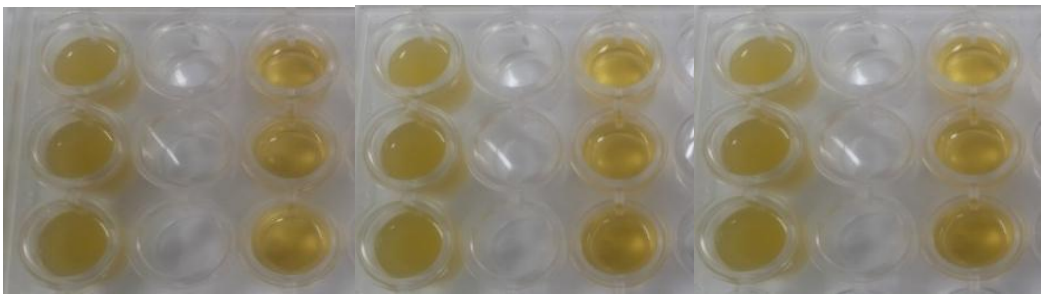
DPPH – radical scavenging assay



Total Phenolic content



Total Flavonoid content



Table(2). Antioxidant Activity of *Acacia concinna*(Willd.) DC. Fruits

No.	Tests	Ethanol extract			Aqueous extract		
		1	2	3	1	2	3
1.	DPPH Antioxidant activity	62.22%	67.56%	66.42%	50.16 %	63.71%	56.56%
2.	Total Phenolic Compound	21.42	23.01	25.14	23.86	25.3	25.44
3.	Total Flavonoid Content	388.08	418.48	403.28	142.48	106.08	113.28

Discussion and Conclusion

In the present study, the morphological characters, phytochemical and antioxidant activities of *Acacia concinna*(Willd.) DC. fruits were described.In morphological characters, *Acacia concinna*(Willd.) DC. is a climbing shrub and a well known medicinal plant widely used in Southeast Asia.The leaves are alternate,

bipinnately compound, paripinnate, stipulate. Inflorescences are cymose; flowers subsessile;calyx campanulate, synsepalous;pods curved and flattened. These characters are in agreement with those mentioned by Hooker(1897), Kitrtikar and Basu(1973) and Dassanayake (1980). The preliminary phytochemical investigation was carried out on the species of *Acacia concinna* (Willd.) DC. fruits. The main constituents of the fruits were found to be alkaloids, glycoside, reducing sugars, α-amino acids, phenolic compounds, saponins, carbohydrates, steroids, tannins, flavonoids and starch. *Acacia concinna* (Willd.) DC. fruits were also studied for the free radical scavenging activity by DPPH assay. Aqueous and ethanolic extracts were prepared and their free radical scavenging activities were evaluated. According to this result, aqueous extracts of fruits showed better scavenging activity than ethanolic extracts. The fruits were possessed considerable amount of phenolic and flavonoid. Therefore, the active compounds of the extracts may be quite polar or hydrophilic.

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