

Investigation of the Physicochemical Properties and Antimicrobial Activity of the Crude Extracts of *Acacia concinna* DC. (Kin-mun-gyin) Leaves

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Abstract

In this research work deals with the study of physicochemical properties and antimicrobial activity of the leaves of *Acacia concinna* DC. (Kin-mun-gyin). The phytochemical investigation of Kin-mun-gyin indicated the presence of alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, terpenoids and tannins whereas cyanogenic glycosides were not detected. The elemental analysis was also carried out by EDXRF and AAS techniques. The percent composition of the nutritional values of this sample was determined by AOAC method. Determination of total ash and physicochemical characterization of this sample were also determined by various soluble matter. Moreover, the antimicrobial activity of polar and non-polar extracts such as pet-ether, ethyl acetate and ethanol extracts from this sample were tested six microorganisms such as pet-ether, ethyl acetate and ethanol extracts from this sample were tested six microorganisms by agar well diffusion method. From these results, it was found that ethanol extract of Kin-mun-gyin leaves showed the most pronounced antimicrobial activity against *staphylococcus aureus* by agar well diffusion method.

Keywords: Phytochemical, nutritional values, physicochemical, antimicrobial activity, agar well diffusion method

1. Introduction

A common, prickly, scandent shrub, occurring in tropical jungles throughout India, especially in the Deccan. *A. concinna* DC. is a medicinal plant that grows in tropical Rainforests of Southern Asia and is also widely distributed in Myanmar Figure 1 [1].



Figure 1. Photographs of the plant and leaves of *Acacia concinna* DC. (Kin-mun-gyin)

2. Materials and Methods

2.1. Sample Collection

The leaves of *Acacia concinna* DC. (Kin-mun-gyin) were collected from Sein Shwe Gyone Ywar, Htauk Kyant, Mingalardon Township, Yangon Region, Myanmar. All the fresh samples were washed with distilled water. After cleaning, the leaves were air-dried at room temperature for three weeks. The dried samples were cut into pieces and then ground in a grinding machine to powder. The dried powdered samples were stored separately in air-tight containers.

2.2. Preliminary Phytochemical Tests

A few grams of dried powdered sample of Kin-mun-gyin was subjected to the test of alkaloids, cyanogenic glycosides, flavonoids, glycosides, phenolic compounds, α -amino acids, reducing sugars, carbohydrates, saponin glycosides, steroids, tannins and terpenoids as the preliminary phytochemical test according to test tube method[2].

2.3. Preparation of Crude Extracts

The dried powdered samples (30 g) were extracted with (150 cm³) of pet-ether, ethyl acetate and ethanol in separate conical flask, respectively for at least 7 days and then filtered. The filtrates were evaporated by using rotatory evaporator and desiccated. Then the dried extracts were weighed. Each extract was stored in refrigerator for screening of antimicrobial activity.

2.4. Determination of Trace Elements and Minerals by AAS and EDXRF

Trace elements and mineral contents from the leaves of *Acacia concinna* DC. were measured at the Universities Research Centre (URC), University of Yangon, Myanmar by applying AAS and EDXRF [3]-[8].

2.5. Determination of Nutritional Values and Physicochemical Properties

Determination of moisture content, protein content, total ash, fiber content, fat content, carbohydrate content, water soluble matter content, 70% alcohol soluble matter content, 95% alcohol soluble matter

content and petroleum ether soluble matter content were carried out in this study [9], [10].

2.6. Screening of Antimicrobial Activity

The antimicrobial activity of different crude extracts (PE, EtOAc and EtOH) were determined against six strains of microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* by employing agar well diffusion method at Botany Department, Kyaukse University, Mandalay Region, Myanmar. [11].

3. Results and Discussion

3.1. Preliminary Phytochemical Tests

From these results it was observed that alkaloids, flavonoids, glycosides, phenolic compounds, α -amino acids, reducing sugars, carbohydrates, saponin glycosides, steroids, tannins and terpenoids were found to be present in sample. But cyanogenic glycosides were not detected in Kin-mun-gyin.

3.2. Nutritional Values and Physicochemical Properties

The experimental work for the nutritional values was carried out at the ministry of co-operatives, Industrial Department, Yangon. According to the experiments, carbohydrate, fat, ash, protein and fiber were found as major constituents. These results from experimental work were shown in Table 1 and Figure 2.

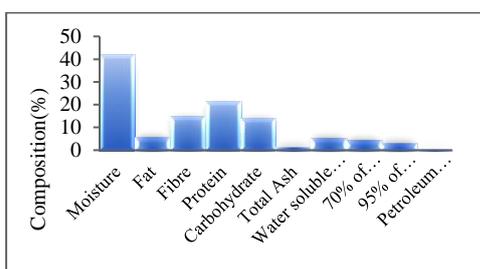


Figure 2. Histogram showing the contents of nutrient values and extractive values of plant sample

Table 1. Results of physicochemical characterization of Kin-mun-gyin leaves

No	Quantity determined	Percent(%)
A		
1	Moisture	42.27
2	Fat	5.606
3	Fiber	15.1
4	Protein	21.47
5	Carbohydrate	14.154
6	Total Ash	1.4
7	Water soluble matter	5.478
8	70% of Alcohol soluble matter	4.474
9	95% of Alcohol soluble matter	3.124
10	Petroleum ether soluble matter	0.436

A = *A.concinna* leaves

Table 2. Content of trace elements in Kin-mun-gyin leaves

No	Sample	Percentage (%)	
		As	Cd
1	Kin-mun-gyin	0.017	0.001

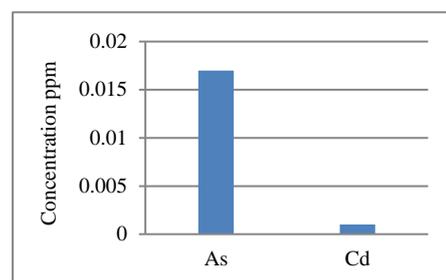


Figure 3. Histogram showing the content of toxic elements (As and Cd) of plant sample

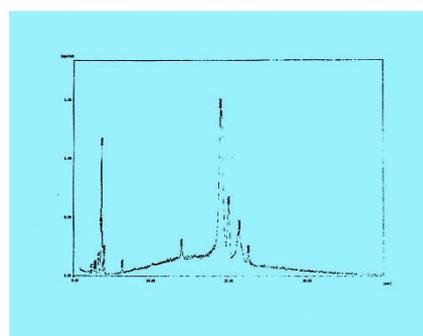


Figure 4 EDXRF spectrum of Kin-mun-gyin leaves

Table 3. Relative composition of some elements in Kin-mun-gyin leaves

Sr. No.	Elements	Relative Composition (%)
1	Ca	68.820
2	K	15.614
3	S	7.876
4	Fe	3.519
5	Mn	2.226
6	Sr	1.946

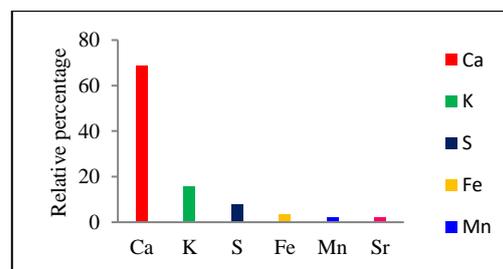


Figure 5. Histogram showing the relative percentages of minerals contained in plant sample

3.3. Elemental Analysis of Plant Sample by AAS and EDXRF

Toxic heavy metals As and Cd are investigated by Atomic Absorption Spectrometer (AAS). The leaves of Kin-mun-gyin were examined for elemental contents

(qualitatively) by EDXRF method. It was found that macronutrients such as Ca, K, and S are present in the leaves of Kin-mun-gyin. Ca is mostly found (68.82 %) in the leaves of Kin-mun-gyin. Other trace elements (micronutrients): Fe, Mn, Sr are also present in Kin-mun-gyin. The results are reported and discussed in Table 2 and 3, Figure 3 and 4.

3.4. Antimicrobial Activity of Crude Extracts by Agar Well Diffusion Method

Screening of antimicrobial activities of various crude extracts such as PE, EtOAc, EtOH of Kin-mun-gyin leaves were investigated by employing agar well diffusion method. In this study, the samples were tested on six species of microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* species. The inhibition zone diameter shows the degree of the antimicrobial activity. The larger the inhibition zone diameters, the higher the antimicrobial activity. The resultant inhibition zone diameters are described in Table 4 and Figure 6.

From these results, it was found that EtOAc and EtOH extract of Kin-mun-gyin leaves showed the most pronounced antimicrobial activity against four microorganisms: *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Candida albicans* with the inhibition zone diameter ranged between 13 mm - 26 mm.

In addition, PE extract (inhibition zone diameter 0 mm), EtOAc extract (inhibition zone diameter 13 mm to 22 mm) exhibited antimicrobial activity against above four species of microorganisms tested. PE extract of Kin-mun-gyin leaves did not show any antimicrobial activity against all organisms tested. But antimicrobial activities of EtOAc and EtOH crude extracts did not show against *Bacillus pumilus* and *Escherichia coli*. Therefore EtOAc and EtOH crude extracts of Kin-mun-gyin leaves can be considered to be biologically active.

Table 4. Antimicrobial activity of different crude extracts of Kin-mun-gyin leaves

No.	Test Organisms	Inhibition Zone Diameter (mm)		
		I	II	III
1	<i>Bacillus pumilus</i> (IFO 905771)	-	-	-
2	<i>Bacillus subtilis</i> (IFO 90571)	-	20.37	21.85
3	<i>Candida albicans</i> (NITE 09542)	-	13.37	14.06
4	<i>Escherichia coli</i> (AHU 5436)	-	-	-
5	<i>Pseudomonas fluorescens</i> (IFO 94307)	-	21.87	22.52
6	<i>Staphylococcus aureus</i> (AHU 8465)	-	22.58	26.67

Agar well diameter = 8 mm

10 mm – 14 mm (low)

15 mm – 19 mm (medium)

20 mm- above (high)

I = PE extract

II = EtOAc extract

III = EtOH extract

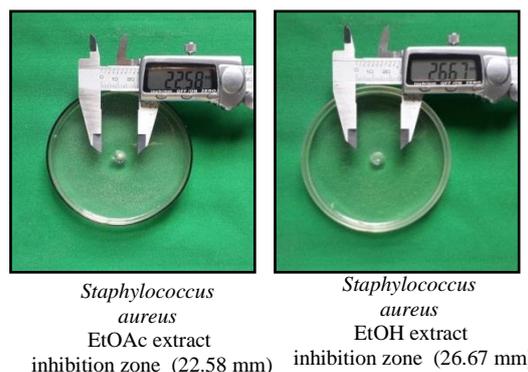


Figure 6. Images of inhibition zones of different crude extracts of Kin-mun-gyin leaves against *Staphylococcus aureus*

4. Conclusion

The following inferences could be deduced from the overall assessment of the chemical investigation on the leaves of *Acacia concinna* DC. (Kin-mun-gyin). Preliminary phytochemical investigation of the Kin-mun-gyin leaves showed that the plant contains alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, terpenoids and tannins. In addition, quantitative determination of the two toxic elements (As, Cd) in Kin-mun-gyin leaves were carried out by atomic absorption spectrometer (AAS). Qualitative elemental analysis of dried powdered leaves sample was done by EDXRF spectrometry. It was found that macronutrients such as Ca, K and S and Fe, Mn and Sr as trace elements are present in the leaves of Kin-mun-gyin. Among these elements, the contents of calcium, potassium and sulphur were 68.820 %, 15.614 % and 7.876 % in the sample as higher constituents. The contents of trace element As and Cd were 0.017 % and 0.001 % in the plant sample. In the nutritional values, the highest amount of protein (21.47 %) and carbohydrate (14.154 %) were found in the powdered leaves sample. As a result, it was found that protein and carbohydrate were present as major nutrients in this sample. Antimicrobial activities of crude extracts (PE, EtOAc and EtOH) of Kin-mun-gyin leaves were screened by using agar well diffusion method against six microorganisms. Except pet ether extracts, ethanol and ethyl acetate crude extracts were found to exhibit the most pronounced antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas fluorescens* and *Bacillus subtilis*, low activity against *Candida albicans*. But antimicrobial activity of all tested crude extract did not show against *Bacillus pumilus* and *Escherichia coli*. In conclusion, it was found that crude extracts of EtOAc and EtOH of Kin-mun-gyin leaves can be effective in the formulation of medicine for the treatment of diseases such as diarrhoea, fever, inflammation, laxative and boils.

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