Antimicrobial and Antioxidant Properties of Crude Extract, and Functional Groups Determination in an Unknown Compound Isolated from Seeds of *Brucea javanica* (L.) Merr

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Abstract

The seeds of *Brucea javanica* (L) Merr were purchased from local Market, Chan Aye Tharsan Township, Mandalay Region, Myanmar. The sample was analyzed for the constituents of phytochemical compounds, such as alkaloid, flavonoid, glycoside, lipophenol, tennin, polyphenol, terpene, steroidand saponin. Moreover, the antimicrobial activity of the sample was examined by Agar-well diffusion method on six organisms such as *Bacillus subtilis, Staphlococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, candida albicans*and *E.coli* respectively. The ethylacetate extract of the seed samples responds to high activities on all tested organisms. Antioxidant activity of seeds of *Brucea javanica* (L) Merr was also determined by DPPH assay. Furthermore, a pure compound would be isolated from the seed sample by applying Thin Layer and Column Chromatographic methods. The constituents of functional groups in the isolated pure compound would be determined by using FT-IR spectroscopic method.

Keyword: Antimicrobial, Antioxidant, *Brucea javanica* (L) Merr, Isolation, FT-IR

Introduction

Bioactive compounds of plants are produced as secondary metabolites.The production of secondary metabolites in different species is mainly selected through the course of evaluation and the particular need of that species. Humans use secondary metabolites as medicines, flavorings and recreational drugs[4].

Some plants having the medicinal value in form of chemical substances that produce a definite physiological action on the human body are called phytochemicals. Since the ancient time these phytochemicals are used to cure the disease in herbal and homeopathic medicines. These are non-nutritive substances, have protective or disease preventive property. There arises a need and therefore to screen medicinal plants for bioactive compounds as a basis for further biomedical studies [3]**.**

The medicinal plants and products are an important source of healthcare in fight against various physical health problems of 85 percent of rural peopleincluding poor in urban area. Some people value these plants due to the ancient belief which says plants are created to supply man with food, medicinal treatment, and other effects. Medicinal plants are the "backbone" of traditional medicine, which means than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. The therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal medicine practitioners. Natural products are the source of synthetic and traditional herbal medicine [6][7].

The plant *Brucea javanica* is considered to be antiperiodic and febrifuge [2]. It is commonly known as lada pahit inMalaysia, is a shrub from the Simaroubaceae family and is widely distributed from Southeast Asia to Northern Australia. A large number of studies on

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this plant have primarily focused on the fruit and seeds, which are commonly used in ethno-medicinal practices for the treatment of lung and gastrointestinal cancers, anti-bacterial and anti-fungal infections, anti-diarrheal treatment of malaria and dysentery, as well as diabetes mellitus. Furthermore, *Brucea javanica*extracts have also been shown to have anti-inflammatory (seeds methanol extract), anti-trypanosomal (fruits ethyl acetate extract) and anti-phytoviral activities (seeds ethanol extract)[8][12].

Brucea javanic (L.) Merr, a Chinese herbal medicine called 'Yadanzi', is distributed in southof China (mainly in Guangxi and Guangdong Provinces) and shows significant antitumor and other activities mostly due to quassinoids, triterpenoids and alkaloids [13].

The bark and roots have been used to treat toothache. The bitterroot is used against the attacks of insects. The seeds are used as an insecticide. In traditional folk medicine, the seed of this plant has been used for the treatment of diabetes and various disorders among indigenous peoples in Malaya peninsula[1][10].

Bioactive compounds contained in plants are vital role of production of medicines. In this research work, seed of *Brucea javanica* (L) Merr, was selected for chemical analysis. In Myanmar, it is known as Yadanzi.

Materials and Methods

The seeds of *Brucea javanica* (L) Merr (Yadanzi) were collected from the local market, Chan Aye Tharsan Township, Mandalay, Myanmar. Phytochemical constituents were screened by phytochemical tests [5]. The antimicrobial activities of the crude samples were determined by using agar well diffusion method at Pharmaceutical and Food Research Department, Insein, Yangon. The antioxidant activity was examined by DPPH assay method[6][7][9]. The dried sample of *Brucea javanica* (L) Merr was percolated with ethanol. The pure organic compounds were separatedby thin layer and column chromatographic method using adsorbent like silica gel 70-230 mesh [3][11]. Then the functional groups present in the isolated compound were estimated by applying FT-IR spectroscopy [10].

Experimental

The dried seeds of *Brucea javanica* (L) Merr (Yadanzi) motored and the fine powder samples would be used for analysis. The phytochemical compounds such as alkaloid, flavonoid, terpene, polyphenol, tannin, lipophenol, steroid and saponin were examined. In antimicrobial activity, the sample extract was tested on six microbial species. These are *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus,Candida albicans* and *E-coli* respectively. According to DPPH assay, antioxidant activity of the sample was carried out by UV spectrometer.

3 grams of ethyl acetate crude extract sample obtained from 105 grams of the dried powder sample. It was separated by thin layer and column chromatographyeluting with normal hexane and ethyl acetate. Among nine combined fractions, fraction IV (yellow oil) and VIII (pale yellow crystal) gave rise to one spot in TLC and then those were recrystallized for purity.Then the polarities of two isolated compounds were compared by the solvent system of column separation. These two compounds were named as compounds I and II.

Results and Discussion

Phytochemical screening showed the content of bioactive compounds in the sample, seed of *Brucea javanica* (L) Merr(Yadanzi).These are alkaloid, flavonoid, glycoside, lipophenol, tennin, polyphenol, terpene, steroidand saponin. The resulds are shown in Table (1).

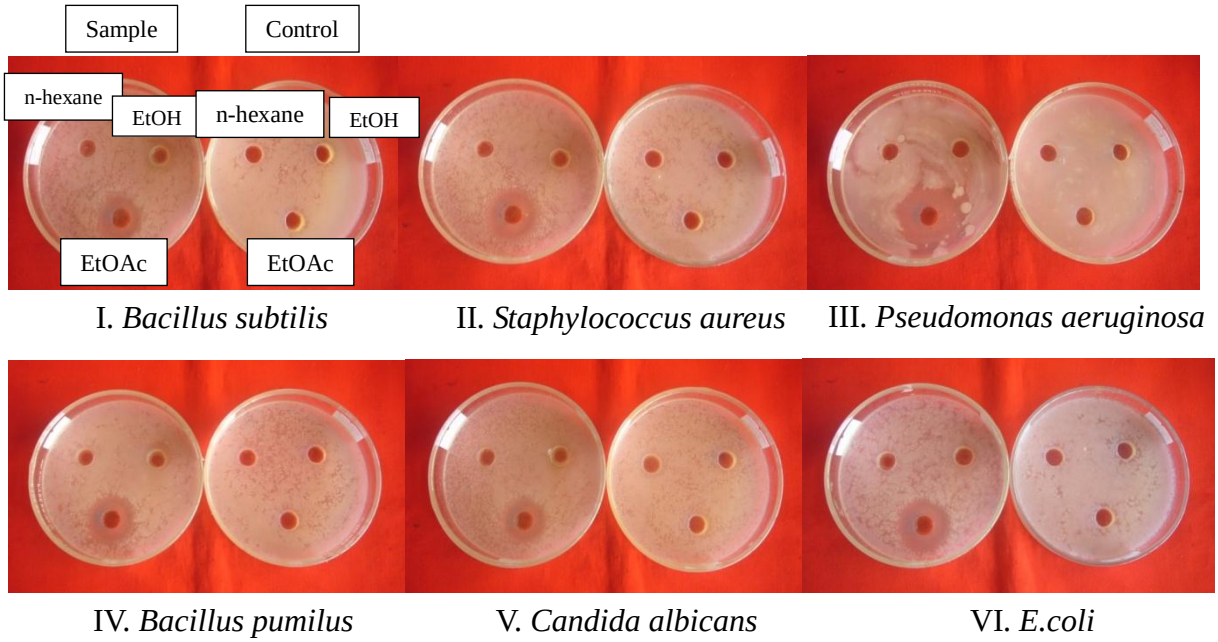
Table (1) Phytochemical compounds contained in *Brucea javanica* (L) Merr (Yadanzi)

No.	Test	Reagents	Observation	Result
1.	Alkaloid	Wagner's reagent	Reddish brown ppt	+
2.	Flavonoid	Mg, conc : HCl	Red color solution	+
3.	Glycoside	10% Lead acetate	yellow ppt	+
4.	Lipophenol	0.5 N KOH, NaOH	Deep yellow color solution	+
5.	Tannin	10% $FeCl_3$, dil H_2SO_4	yellowish brown ppt	+
6.	Polyphenol	1% $FeCl_3$ and 1% K_3 [Fe $(CN)_6$]	Greenish blue color solution	+
7.	Terpene	Acetic anhydride, Conc. H_2SO_4, $CHCl_3$	red ppt	+
8.	Steroid	Acetic anhydride, Conc. H_2SO_4, pet-ether	green color solution	+
9.	Saponin	Distilled water	frothing	+

According to the antimicrobial activity tests, ethyl acetate extract of the crude sample gave highest activity on all tested organisms. Ethyl acetate is one of the polar solvents. It means that bioactive compounds are more soluble in polar solvent. The ethyl acetate extract of sample was found the highest inhibition on *Pseudomonas aeruginosa*.

Table (2) Antimicrobial Activities of seed of *Brucea javanica* (L) Merr

Samples	Solvents	Inhibition zone					
		I	II	III	IV	V	VI
Yadanzi	EtOAc	20 mm (+++)	23 mm (+++)	35 mm (+++)	25 mm (+++)	20 mm (+++)	25 mm (+++)
	EtOH	13 mm (+)	13 mm (+)	11 mm (+)	13 mm (+)	13 mm (+)	13 mm (+)
	n-hexane	-	-	-	-	-	-



I. *Bacillus subtilis* II. *Staphylococcus aureus* III. *Pseudomonas aeruginosa*

IV. *Bacillus pumilus* V. *Candida albicans* VI. *E.coli*

Figure (1) Antimicrobial activity of seed of *Brucea javanica on* six organisms

Table (3) Antioxidant Activity of seed of *Brucea javanica* (L) Merr

Extract	**Concentration**	**Mean Absorbance**	**Mean(%) Inhibition**	**IC_{50} (µg/mL)**
Ethanol extract sample	100 90 80 70 60	0.441 0.512 0.529 0.551 0.598	52.78 45.18 43.36 41.00 35.97	96.79
Ascorbic acid	50 25 12.5 6.25 3.125	0.297 0.350 0.483 0.562 0.608	68.50 61.61 48.78 40.41 35.52	17.99

Antioxidant activity of the sample was found as a low activity than ascorbic acid. IC_{50} value of the sample was 96.79 µg/mL. In ascorbic acid, it was observed as 17.99 µg/mL. The results of antioxidant activity were described in Table (1), Figure(2) and Figure (3).

The two pure compounds were separated from the column. Among these two compounds, compound II was separated from more polar solvent system. It may be soluble in polar solvents such as ethyl acetate and ethanol.

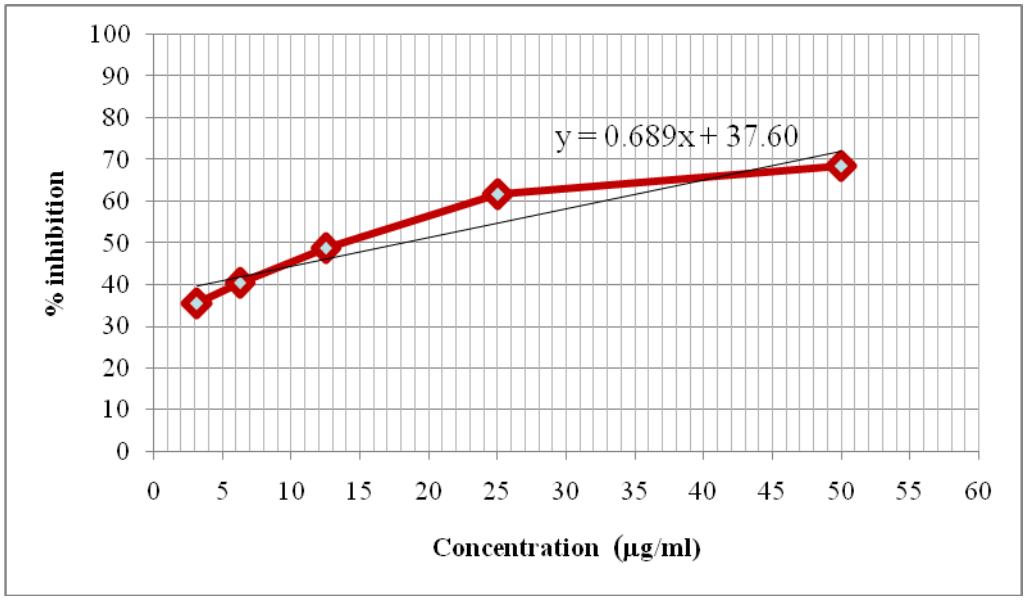


Figure (2) Plot of % Inhibition Vs Absorbance of Ascorbic Acid

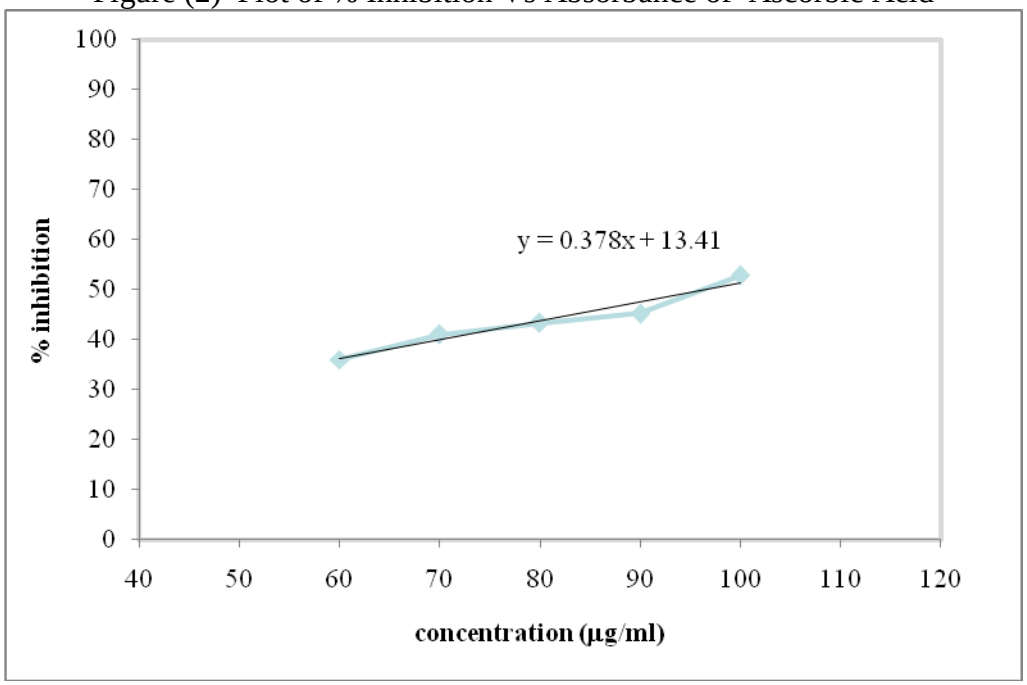


Figure (3) Plot of % Inhibition Vs Absorbance of Sample

The yield % of isolated compound II is 0.166 % (4.98 mg) and it was observed as white crystal form. In accordance with the results of antimicrobial activity, the isolated pure compound II was chosen for functional groups determination by FT-IR spectroscopic technique. The FT-IR spectral data informed the functional groups present in the isolated pure compound II.The broadband was found at 3369.75 $cm^{-1}$for hydroxyl groups. The bands appeared at 3099.71 cm^{-1}, 2928.04 cm^{-1} and 2858.60 cm^{-1} indicated the constituents of sp^2 hydrocarbons and sp^3 hydrocarbons.

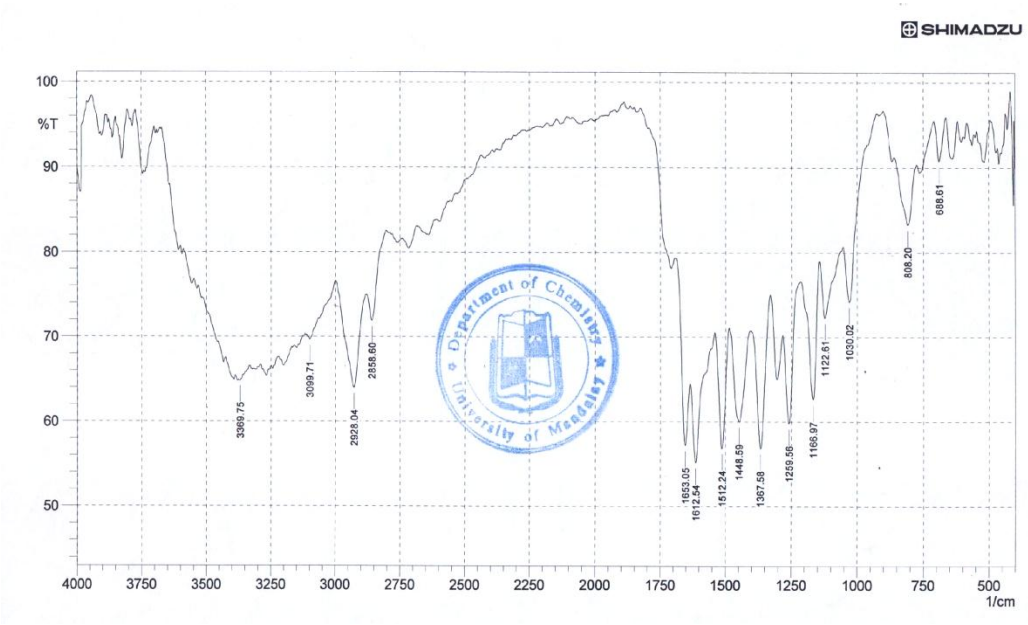


Figure (4) FT-IR Spectrum of the Isolated Compound II from *Brucea javanica*(L) Merr

The sharp absorption band at 1653.05 cm^{-1}showed the presence of carbonyl group in this compound.In addition, C=C ring skeleton , C-H in plane bending vibration, C-O-C stretching vibration, C-O stretching vibration , C-CO-OC stretching vibration and C-H out of plane bending vibration of Trans or E and Cis or Z alkenic groups would be observed at 1612.54 cm^{-1}, 1512.24 cm^{-1}, 1448.59cm^{-1}, 1367.58cm^{-1}, 1259.56 cm^{-1}, 1166.97 cm^{-1}, 1122.61cm^{-1}, 1030.02 cm^{-1} and 808.20 cm^{-1} respectively.

Conclusion

In this research work, the seeds of *Brucea javanica* (L.) (Yadanzi) were selected for the isolation and functional groups determination of pure bioactive compound by applying column and thin layer chromatographic method. According to the phytochemical screening, it consists of alkaloid, flavonoid, glycoside, lipophenol, tennin, polyphenol, terpene, steroidand saponin are present. As tabulated in Table (2), The ethyl acetate extract of the seeds highly inhibited on all tested organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E-coli* respectively.Especially, ethyl acetate extract was the highest activity on *Pseudomonas aeruginosa.* N- hexane extract did not against all tested organisms.IC_{50}value of ascorbic acid was 17.99µg/mL and IC_{50}value of sample is 96.79µg/mL by determination of antioxidant activity using DPPH assay.Therefore, the sample has less antioxidant activity than ascorbic acid.According to the FT-IR spectral data of isolated compound II, the hydroxyl groups, sp^2 hydrocarbons of alkenic groups, asymmetric and symmetric sp^3 hydrocarbons, carbonyl group, allylic hydrocarbons and ether group are present in this compound.

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