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Antimicrobial Activity and Chemical Constituents of the Extract from *Jatropha curcas* Fruit

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ABSTRACT

The antimicrobial activity and chemical constituents of the methanolic extract from *J. curcas* fruit were evaluated in this study. The crude extract was achieved by extraction with 60 % (v/v) methanol. It showed the potencies of antimicrobial activity against *P. putida*, *P. syringae* pv. *sesami*, *X. campestris*, *X. campestris* pv. *glycines*, *X. campestris* pv. *vesicatoria* and *R. solanacearum* with the presence of inhibition zone in the range of 8.0 ± 0.0 to 13.7 ± 0.6 mm and MIC value at 214.29 ± 0.00 µg/mL. Furthermore, flavone compound can be proposed by the analysis of gas chromatography-mass spectrometry (GC-MS). According to the group of flavonoid compounds have strong bioactive properties; the results suggested that *J. curcas* fruit has highly potential as effective natural bioactive sources.

Keywords: *Jatropha curcas* fruit, Antimicrobial activity, Disc diffusion method, Broth micro-well dilution method, GC-MS analysis.

INTRODUCTION

Polyphenolic compounds such as flavonoids, coumaric acids and tannins possess many biological activities which are attributed to their antimicrobial activity[1,2]. These compounds also demonstrate antiviral, anti-inflammatory and anticancer properties[3]. Especially, flavonoids are well known as polyphenolic substances with strong bioactive activity which obtained from many types of medicinal plants[4]. However, in currently the synthetic bioactive compounds such as butylatedhydroxyanisole(BHA), butylatedhydroxytoluene (BHT) and tert-butylhydroquinone are usually used in industrial process[3,5,6]. The synthetic antimicrobial chemicals are also used in the treatment of infectious diseases. However, these compounds have been considered the toxic and carcinogenic effect which can cause serious disease to human body[7]. Accordingly, the importance of searching for natural antimicrobial compounds for replacement the using of synthetic compounds has increased significantly in the present.

One of medicinal plants which are interesting for the natural antimicrobial compounds

elucidation is *Jatropha curcas* (*J. curcas*). It is a multipurpose plant in *Euphorbiaceae* family which has a lot of economic significance, especially for biodiesel production source and also its medicinal values[8,9]. Numerous parts of *J. curcas* plant have been studied about phytochemical compound identification for searching new natural bioactive compounds in previously reported[8,10]. The parts of *J. curcas* plant such as roots, stems, barks, leaves, seeds, which can be used for various purposes including medicinal value[11,12]. Accordingly, the studies about finding natural bioactive compounds from other parts of *J. curcas* plant are interesting. This study focuses on the antimicrobial activity of chemical constituents from whole fruit of *J. curcas*, which has no report available on the study of the entire *J. curcas* fruit in previous research. The *J. curcas* green fruits were selected to study in this work, as shown in Figure 1.

Therefore, the aims of the present study were to evaluate the antimicrobial activity of methanolic extract from *J. curcas* fruit. Additionally, gas chromatography-mass spectrometry (GC-MS) was used to identify the chemical constituents in methanolic extract of *J. curcas* fruit. The achieved information would indicate the possible of the *J. curcas* fruit extract as a new source of natural bioactive compounds. Especially, there are plentiful *J. curcas* plants in Thailand. In order to add value of the local plants besides the biodiesel production, the study is important.

Fig. 1: The *Jatropha curcas* fruits

MATERIALS AND METHODS

J. curcas fruit was kindly supported by Khon Kaen Field Crops Research Center (KKFCRC)Khon Kaen Province, Thailand. The analytical grade of organic solvent including methanol,ethanol, hexane, dichloromethane, chloroform and ethyl acetate were purchased from Carlo Erba (Italy). The analytical grade of dimethyl sulfoxide (DMSO) was obtained from RCI Labscan (Thailand). The culture media including Nutrient agar (NA), Nutrient broth (NB), Agar powder, Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) were purchased from Himedia (India). Gentamicin sulfate salt hydrate was also obtained from Sigma-Aldrich (Switzerland). A Supelclean™ LC-Si SPE cartridge (6 mL, 500 mg) was purchased from Supelco (USA).

Bacterial strains

The microorganisms for antimicrobial activity evaluation including seven plant pathogenic bacteria, there are *P. putida*, *P. syringae* pv. *sesami*, *X. campestris*, *X. campestris* pv. glycines, *P. syringae*, *X. campestris* pv. *vesicatoria* and *R. solanacearum*. All microorganisms were obtained from Microbiological Resources Center, Bangkok, Thailand Institute of Science and Technological Research (TISTR), Thailand.

The extraction of *J. curcas* fruit

The extraction was conducted according to the method of Oskoueian *et al.,* (2011) with slight modification[1].Briefly, powdered *J. curcas* sample (10 g) was extracted three times with 50 mL of 60% (v/v) methanol by shaken with an orbital shaker (Yamato Scientific, Japan) at 150 rpm at ambient temperature (30 °C) for 2 h. The extract was then filtered through a Whatman No. 1 filter paper. Afterward, combined filtrates were evaporated by rotary vacuum evaporation. The obtained crude extracted solution was further fractionated by loading through a Supelclean™ LC-Si SPE cartridge and eluted with 25 mL of hexane, dichloromethane, chloroform, ethyl acetate and methanol, respectively. The solvent of each fraction was removed by rotary vacuum evaporation and the obtained remainder defined as F_1, F_2, F_3, F_4 and F_5, respectively.

Determination of antimicrobial activity

Disc diffusion method

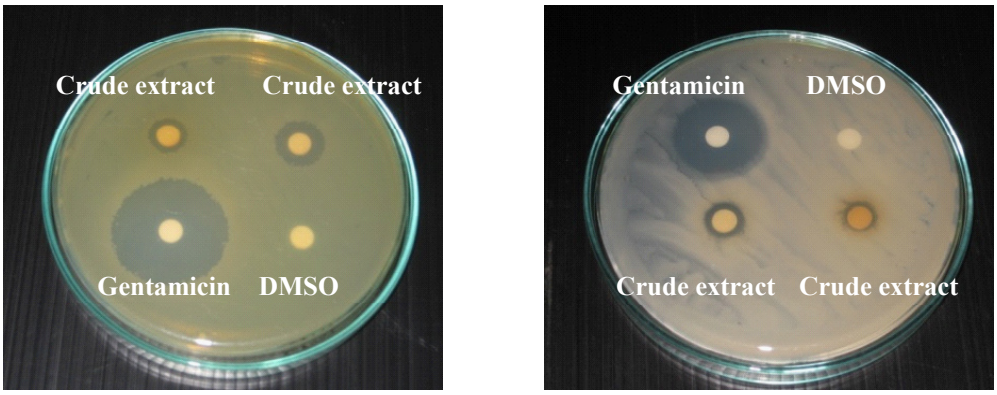
In order to evaluate the antimicrobial activity of crude methanolic extract from *J. curcas* fruit, the disc diffusion method was selected according to the reported of Gulluce *et al.*, (2007) with small modification[13]. The seven plant disease bacteria were evaluated. Firstly, each test bacterial strain was grown in nutrient broth (NB) liquid medium at 37 °C for 24 h.The obtained inoculum was then diluted with sterile water to achieve the optical density (OD_{600}) equal to McFarland No. 0.5 (1.5 x 10^8 CFU/mL). Afterward, 100 µL of this bacterial solution was spread on the Mueller-Hinton agar (MHA) in Petri dishes and allowed to dryness. Finally, sterile paper discs with crude methanolic extracts were carefully applied on the surface of the preparing agar plate which containing tests microorganism. Gentamicin was used as positive control and negative control was also set up by the equal quantity of DMSO. These obtained agar plates were then incubated at 37 °C for 24 h and the antimicrobial activity was evaluated by measuring the inhibition zone. The experiments were performed in triplicate.

Broth micro-well dilution method

For evaluate the minimum inhibitory concentration (MIC) value of the extracts the broth micro-well dilution method was performed as the report of Kivrak *et al.*, (2009)[14]. Briefly, the highest concentration of crude methanolic extract (1000 µg/mL) was prepared two-fold serial dilutions in Mueller-Hinton Broth (MHB). 75 µL of the culture media (Mueller-Hinton Broth) was added into each hole of 96-well microplates. 100 µL of the initially concentration of crude methanolic extract at 1000 g/µL were added into the first raw of microplate. Afterward, their serial dilutions were prepared by

Table 1: The Antimicrobial Activity of Crude Methanolic Extract From *J. curcas* Fruit

Plant Disease Bacteria	Antimicrobial Activity	
	Inhibitionzone (mm)	MIC (µg/mL)
Pseudomonas putida	13.7 ± 0.6	214.29 ± 0.02
Pseudomonas syringae pv. sesami	10.0 ± 0.0	428.57 ± 0.04
Xanthomonas campestris	8.0 ± 0.0	214.29 ± 0.00
Xanthomonas campestris pv. glycines	8.3 ± 0.6	214.29 ± 0.00
Pseudomonas syringae	inactive	inactive
Xanthomonas campestris pv. vesicatoria	8.7 ± 0.6	214.29 ± 0.02
Ralstonia solanacearum	8.3 ± 0.6	428.47 ± 0.02



(a) (b)

Fig. 2: Disc diffusion inhibition zone of (a) *P.putida,* (b) *X.campestrispv. glycines*

transferring 100 µL of solution into consecutive wells. Each microorganism was grown in nutrient broth (NB) liquid medium by incubation at 37 °C for 24 hrs. The inoculation of bacteria was prepared by dilution with sterile water until the bacterial cell equal to McFarland No. 0.5 (1.5 x 10^8 CFU/mL). And then, 25 µL of the inoculum was added into each raw of microplate. These solutions were mixed and incubated at 37°C for 24 h. Finally, the absorbance at 655 nm was recorded by using a microplate reader. Gentamicin was also used as positive control for antimicrobial activity. The experiments were performed in triplicate.

Identification the chemical constituents of the extracts using GC-MS

In order to identify the extracted compounds which contain in the extracts, a Thermo Finigan Polaris Q mass spectrometer (USA) coupled to an Agilent trace GC chromatograph were used. The separation of the analytes was performed on a methyl silicone HP-5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm film thicknesses). Briefly, 1 µL of the extracts was injected into the gas chromatograph using splitless mode. The carrier gas was helium with flow rate 1 mL min^{-1}. The extracts in methanol were injected at a column oven temperature of 70 °C, in which this temperature was programmed to increase from 70 °C-280 °C at 10 °C/minutes. The transfer line temperature was at 275 °C and the ion source temperature maintained at 200 °C. Electron impact (EI) mass spectra (70 eV) of the parent compounds were recorded. A mass range of m/z 50-650 was scanned, data attained and processed by using theXcalibur software version 1.3.

Statistical analysis

Statistical analysis was carried out using Microsoft Corporation Computer Excel Program (USA). All experiments were performed in triplicate. The results were presented as a value ± standard deviation of mean (SD).

RESULTS AND DISCUSSION

Antimicrobial activity of the crude methanolic extract

The activity of crude methanolic extracts from *J. curcas* fruit on seven plant disease bacteria were evaluated by Disc diffusion method and Broth micro-well dilution method. The results showed that the extract indicated the potencies of antimicrobial activity against six plant disease bacteria including *P. putida*, *P. syringae* pv. *sesami*, *X. campestris*, *X. campestris*pv. *glycines*, *X. campestris*pv. *vescicatoria* and *R. solanacearum* as shown in Table 1.This extract exposed antimicrobial activity effect against six plant disease bacteria with inhibition zone in the range of 8.0 ± 0.0 to 13.7 ± 0.6 mm and moderate antimicrobial activity with MIC value at 214.29 ± 0.00 µg/mL. The instance of antimicrobial activity of this extract against some tests bacterial strain including *P. putida* and *X. campestris* pv. *glycines* were shown in Fig. 2.

As the results, the crude methanolic extract showed lower antimicrobial activities than of that positive control, gentamicin. It exposed antimicrobial activity effect with varying degrees of growth inhibition zone against the test bacterial strains. For the selected criteria of antimicrobial activity evaluation, the extract with MIC less than75 µg/mL was considered to have strong antimicrobial activity, MIC 75-150 µg/mL, the antimicrobial activity was moderate and MIC more than 250 µg/mL the antimicrobial activity was considered inactive[15].

Consequently, the crude methanolic extract as this work revealed moderate antimicrobial activity against *P. putida*, *X. campestris*, *X. campestris* pv. glycines, *X. campestris* pv. *vesicatoria*. The obtained antimicrobial activity may correspond with the bioactive compounds which contained in the medicinal plant such as phenolic and flavonoid compounds as described in previous report[16-18]. For instance, Bounatirou *et al*., (2007) described that the main components in studied plant extract that showed the antimicrobial activity were terpene phenols[19]. Moreover, as the study of Hamed *et al*., (2015), two flavonol compounds exhibited significant antimicrobial effect against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* bacteria[20]. However, this activity may be due to the content of phorbol ester. In which many of reports described that phorbol ester containing in high quantity in the part of *J. curcas* seed[5]. According to the obtained antimicrobial activity in this study, the chemical constituent in the crude methanolic extract from *J. curcas* fruit can be served as an effective natural antimicrobial source.

GC-MS analysis of the extract from *J. curcas* fruit

The crude methanolic extract of *J. curcas* fruit was fractionated by using a Supelclean™ LC-Si SPE cartridge and the fractions specify as F_1, F_2, F_3, F_4 and F_5 were obtained. The yellow solution of methanolic fraction (F_5) was selected to identify by GC-MS due to the high quantity of this fraction was achieved. The mass spectrum of interested compound (RT = 11.38) with the predominant fragment at m/z 223.44 and m/z 327.35 was shown in Fig. 3. The type of phenolic compound was tentatively identified on the basis of retention time and mass pattern, as well as comparing into the dada in related literature. However, there is no report available in the literature on the characterization of the methanolic extract from *J. curcas* fruit. The compound may be proposed that this extract contain the component of some flavonoid compound due to the pattern of flavone skeleton was identified. Consequently, it is possible that their antimicrobial activity of this methanolic extract may be due to the containing of the proposed compound, flavone. Importantly, many case of flavonoid compound show strong antimicrobial activity in the literature reported[21,22].

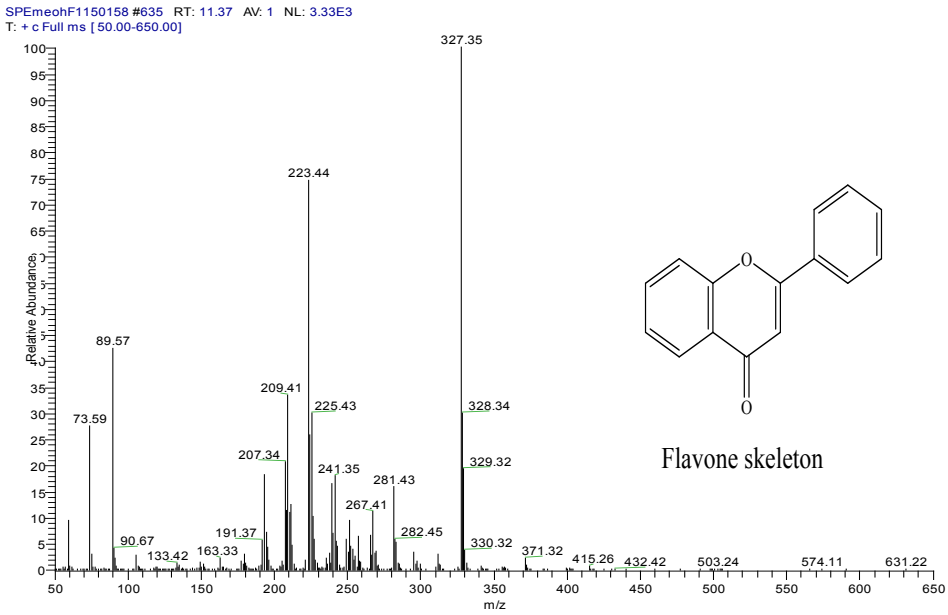


Fig. 3: The mass spectrum of methanolic extract from *J. curcas* fruit at RT = 11.38

CONCLUSIONS

According to many of researches required about management of *J. curcas* as to know more about the utilization of its products. Especially, there are plentiful *J. curcas* plants in Thailand due to the encouragement of *J. curcas* cultivation increase for biodiesel production source over the last several years. Consequently, this research was performed in order to support these purposes by evaluating the utilization of other part of *J. curcas* such as its fruit part. It was found that the of *J. curcas* fruit gave antimicrobial activities against *P. putida*, *P. syringae* pv. *sesami*, *X. campestris*, *X. campestris* pv. glycines, *X. campestris* pv. *vesicatoria*, *R. solanacearum*. The chemical constituent analysis by GC-MS technique demonstrated that flavone compound can be proposed. The results confirm that *J. curcas* fruits are the source of some important bioactive compounds which corresponding the antimicrobial properties and may be useful for medicinal application in the future.

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