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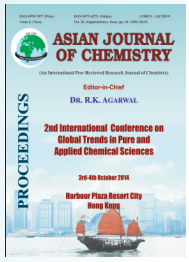

Antioxidant and Antimicrobial Activities of Methanolic Extract from *Jatropha curcas* Linn. Fruit

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The present study was designed to evaluate the antioxidant activity and also antimicrobial activity with total phenolic content of the crude methanolic extract from *J. curcas* Linn. fruit. The dried samples were extracted by 60 % (v/v) methanol to obtain the crude methanolic extract. The total phenolic content of the crude methanolic extract which was analyzed by Folin-Ciocalteu method was 7.04 ± 0.10 mg/g as GAE. The evaluation of antioxidant activity of the crude methanolic extract was performed by using two methods including 2,2-diphenyl-1-picryhydrazyl and Phen assay. The results showed that the antioxidant activity of this extract was 270.98 ± 0.59 µmol Fe/g of extract for Phen assay and the extract gave IC_{50} at 14.09 ± 0.05 mg/mL for 2,2-diphenyl-1-picryhydrazyl assay. Additionally, as the antimicrobial activity evaluation, this extract exhibited 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. All test methods showed that the crude methanolic extract from *J. curcas* Linn. fruit had a higher total phenolic content and antioxidant activity than *J. curcas* seed oil in previous work, while the antimicrobial activity was moderated. Therefore, these results suggested that *J. curcas* Linn. fruit has highly potential as effective natural bioactive sources.

Keywords: *Jatropha curcas* Linn. fruit, Total phenolic content, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

Jatropha curcas Linn. (*J. curcas*) is a multipurpose plant in the family of *Euphorbiaceae* with many attributes and considerable potential energy crops[1]. Various parts of the plant have been widely used in difference applications in previous studies[2-4]. The *J. curcas* seed oil has been considered promising as a material for biodiesel production[5]. Moreover, the other parts of this plant such as roots, stems, barks, leaves, seeds and fruits can be used for numerous purposes including medicinal value[6-8]. Oskoueian *et al.*[8] reported that the extracts of root and latex of *J. curcas* which contain phenolic compounds showed notable bioactive activities such as antimicrobial, antioxidant and anticancer. Consequently, the studies about finding natural bioactive compounds from other parts of this plant are interested.

Various antioxidant activity tests have been used to evaluate the antioxidant activity in previous researches, such as 2,2-diphenyl-1-picryhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP) assay and phenanthroline (Phen) assay[9-12]. For the reasons that the various compounds with different chemical behaviour are diversity, the use of more than one method for antioxidant activity evaluation is highly suitable[13]. Several reports described that phenolic compounds are main substances that have influence of antioxidant activity in plant extracts[10,14,15]. Fu *et al.*[3] studied the correlation between total phenolic content and antioxidant activity of *J. curcas* seed shell using Folin-Ciocalteu and seven different antioxidant methods, 2,2-diphenyl-1-picryhydrazyl, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) [ABTS], OH radicals scavenging, oxygen radical absorbance capacity (ORAC), reducing power, metal chelating and β-carotene bleaching assays. The results indicated that the amounts of total phenolic content correspond to antioxidant activities.

Several methods have been reported for evaluation of antimicrobial activity of plant extracts including disc diffusion method and broth micro-well dilution method[8,9]. Tongpoothorn *et al.*[9] studied bioactive properties of methanolic extract and its fraction from *J. curcas* oil by determining total phenolic content and antimicrobial activities[9]. Nithiyanantham *et al.*[16] reported that *J. curcas* was a potential source of natural anti-microbial and could be a good candidate for pharmaceutical plant based products. Because of the possible toxicities of the synthetic antimicrobial drugs, increasing attention has been directed toward the natural antimicrobial compounds[10]. This research focuses on the biological activities of chemical

constituents from whole fruit of *J. curcas*, which has no report available on the study of entire *J. curcas* fruit in previous studies. The *J. curcas* green fruits were selected to study in this work, as shown in Fig. 1.

Fig. 1. *Jatropha curcas* fruits

Accordingly, the present study is interested in the correlation of bioactive properties and phenolic compounds containing in *J. curcas* fruit. Therefore, the aims of this research were to evaluate the bioactive properties of the extract of *J. curcas* fruit by determining total phenolic content, antioxidant and antimicrobial activities. The information gathered would indicate the possible of the *J. curcas* fruit as a source of natural bioactive compounds.

EXPERIMENTAL

J. curcas fruit was supported from Khon Kaen Field Crops Research Center (KKFCRC) Khon Kaen Province, Thailand. The analytical grade of dimethyl sulfoxide (DMSO) was purchased from RCI Labscan (Thailand). Folin-Ciocalteu reagent was obtained from merck (Germany). 2,2-diphenyl-1-picryhydrazyl (DPPH), gallic acid, 1,10-phenanthroline and gentamicin sulfate salt hydrate were obtained from Sigma-Aldrich (Switzerland). 2,6-di-*tert*-butyl-4-methylphenol (BHT) was purchased from acros organic (USA). Ferric chloride, sodium carbonate, sodium hydroxide and ferrous sulfate heptahydrate, methanol and ethanol were purchased from Carlo Erba (Italy). The culture media for antimicrobial activity study including nutrient agar (NA), Nutrient broth (NB), Agar powder, Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) were purchased from Himedia (India).

Microorganisms: The test bacterial strains including seven plant pathogenic bacteria, there are *Pseudomonas putida*, *Pseudomonas syringae* pv. *sesami*, *Xanthomonas campestris*, *Xanthomonas campestris* pv. *glycines*, *Pseudomonas syringae*, *Xanthomonas campestris* pv. *vesicatoria*, *Ralstonia solanacearum*. All strains were purchased from Microbiological Resources Center, Bangkok, Thailand Institute of Science and Technological Research (TISTR), Thailand. The stock cultures of all bacteria were maintained on NA at 4 °C. The test bacterial strain was activated the growth on NA plate and incubated at 37 °C before further study.

Extraction of *J. curcas* fruit: *J. curcas* fruits were cut into small size and dried in an oven at 50 °C for 48 h. The dried sample was then pulverized using an electric blender. The extraction procedure was carried out according to the process of Oskoueian[8] with minor modification. Briefly, 10 g of *J. curcas* dried sample was extracted with 50 mL of 60 % (v/v) methanol by shaken with an orbital shaker (Yamato Scientific, Japan) at 150 rpm at ambient temperature for 2 h. After that, the extract was filtered through a Whatman No. 1 filter paper. Subsequently, the solvent in the filtrate was evaporated by rotary vacuum evaporation and then crude methanolic was obtained.

Determination of total phenolic content (TPC): Total phenolic content of *J. curcas* fruits were determined by using Folin-Ciocalteu method described by Ganesan *et al.*[15] with slight modification. Firstly, 200 µL of the extracts or standard solution of gallic acid was added into a test tube. After that, 1.5 mL of 10 fold diluted Folin-Ciocalteu reagent was added. And then, 3 mL of 7.5 % (w/v) sodium carbonate was added and adjusted volume into 10 mL with distilled water. The mixtures were kept in the dark at ambient temperature. After 0.5 h, the absorbance of the reaction mixtures was measured at 765 nm by using a UV-visible spectrophotometer (Agilent model 8453, Germany). The experiments were carried out in triplicate. Standard gallic acid was used for calibration curve. The results were reported as gallic equivalent (GAE) in mg per 1 g of the extract.

Evaluation of antioxidant activity

2,2-Diphenyl-1-picryhydrazyl (DPPH) method: Antioxidant activity of the crude methanolic extract was evaluated using 2,2-diphenyl-1-picryhydrazyl method followed as the report of Tongpoothorn *et al.*[9] with slight modification. Stock solution of 2,2-diphenyl-1-picryhydrazyl radical was freshly prepared in methanol. The extracts or standard BHT were added with 100 µM 2,2-diphenyl-1-picryhydrazyl and adjusted volume to 10 mL with methanol. The solutions were mixed and incubated at ambient temperature for 0.5 h. Absorbance of control, sample and BHT were measured at 517 nm by using a UV-visible spectrophotometer. The experiment was carried out in triplicate. The results were reported as IC_{50} value, which obtained from the plotting of % inhibition (eqn.1) against concentration of the extracts.

$$\% \text{ Inhibition} = \frac{A_{517} \text{ control} - A_{517} \text{ sample}}{A_{517} \text{ control}} \times 100 \quad \text{(i)}$$

Phenanthroline (Phen) method: Antioxidant activity of the crude methanolic extract was also determined by Phenthroline method as described by Szydlowska-Czerniak *et al.*[13] with minor modification. Briefly, each extract was mixed with 0.2 % (w/v) of ferric chloride. Afterward, 0.5 % (w/v) of 1,10-phennanthroline solution in methanol was added and adjusted volume to 10 mL with methanol. The obtained solutions were mixed and incubated at ambient temperature. After 0.5 h, the absorbance of mixture was measured at 510 nm against blank. Ferrous sulfate solutions were used to prepare for calibration curve. This method was reported amount of antioxidant activity on µmoL of Fe per 1 g extract. These processes were studied in triplicate. Total phenolic content and both antioxidant activity of the extracts were expressed as the mean ± standard deviation (SD).

Evaluation of antimicrobial activity

Disc diffusion method: Antimicrobial activity of the crude methanolic extracts from *J. curcas* fruit were evaluated by disc diffusion method according to the reported of Gulluce *et al.*[17] with minor modification. The seven economic plant pathogenic bacteria were studied. The tests bacteria were grown in nutrient broth (NB) liquid medium at 37 °C for 24 h. This inoculum was diluted with sterile water until the optical density (OD600) equal to McFarland No. 0.5 (1.5×10^8 CFU/mL). Subsequently, this solution was spread on the Mueller-Hinton agar (MHA) plate and allowed to dryness. Sterile paper discs with crude extracts were applied to the agar plate which containing tests bacteria. The agar plates were incubated at 37 °C for 24 h and evaluated by measuring the zone of inhibition. These studies were conducted in triplicate.

Broth micro-well dilution method: The broth micro-well dilution method was performed for the evaluation of minimum inhibitory concentration (MIC) value[18]. The first concentration of crude extracts solution were prepared two-fold serial dilutions in mueller-hinton broth (MHB). The 96-well micro plates were prepared by added 75 µL of the culture media into each well. 100 µL of the first crude extracts were added into the first well. Then, 100 µL of solution were transferred into consecutive wells for preparation of their serial dilutions. The tests bacteria were grown in nutrient broth (NB) liquid medium at 37 °C for 24 h. The inoculum was diluted with sterile water until the bacterial cell equal to McFarland No. 0.5 (1.5×10^8 CFU/mL). After that, 25 µL of the bacterial solution was added into each well. After mixing, all microplates were incubated at 37 °C for 24 h. Finally, the absorbance was detected at 655 nm by using a microplate reader. Gentamicin was used as a reference compound for antibacterial activities. The MIC was defined as the lowest concentration of the crude extracts to inhibit the growth of bacterial strain. All experiments were carried out in triplicate and results were expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Total phenolic content of the extracts: The Folin-Ciocalteu method was used to evaluate total phenolic content of the extracts from *J. curcas* fruit. The reaction of Folin-Ciocalteu method was proposed that a complex was formed between phosphomolybdic tungstate and the reducing species from the extracts. Consequently, the colour of solution change from yellow to blue and the absorbance at 765 nm was observed[19]. Total phenolic content of the extracts were assessed to establish their effect on bioactive properties, antioxidant and antimicrobial activities. Total phenolic of the extracts were determined as mg of gallic acid equivalent (GAE)/g of the extract by using the regression equation from the calibration curve of gallic acid standard in the range of 25-250 µg/mL (Fig. 2). The results showed that Total phenolic of crude methanolic extracts was 7.04 ± 0.10 mg GAE/g of the extract (Table-1). In addition, the obtained quantity of crude methanolic extract was also shown in Table-1.

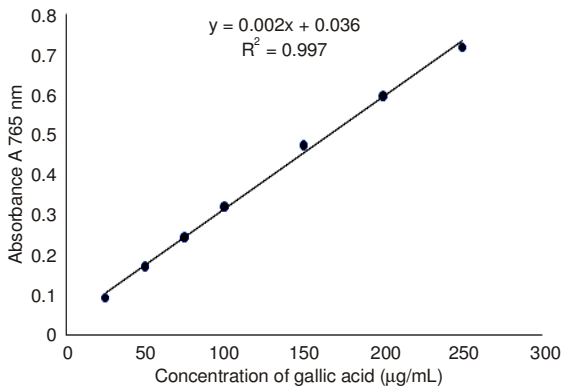


Fig. 2. Calibration curve of gallic acid standard in Folin-Ciocalteu method

Antioxidant activity of the extracts: The evaluation of antioxidant activity by more than one method is advantage due to different methods assess different characteristics of antioxidant compounds[19]. Antioxidant activity of the methanolic extracts from *J. curcas* fruit was evaluated by two methods with different mechanisms including 2,2-diphenyl-1-picryhydrazyl assay based on free radical scavenging while Phen assay based on reduction of ferric ion by antioxidant compounds. As Phen method, calibration curve of ferrous sulphate in the range of 100-700 µmol/L was obtained (Fig. 3). The crude methanolic extract demonstrated antioxidant activity 270.98 ± 0.59 µmol Fe/g of the extract (Table-1).

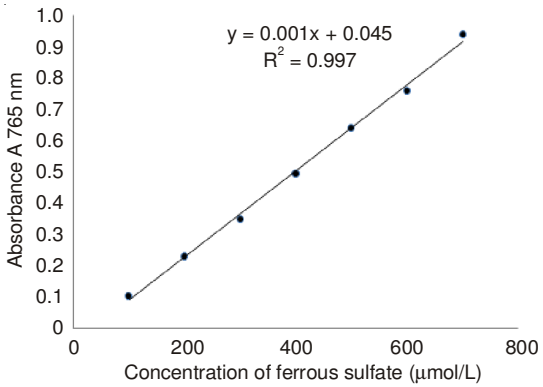


Fig. 3. Calibration curve of ferrous sulfate obtained from Phen assay

It was found that the highly antioxidant activities as this assay correlated with total phenolic content by Folin-Ciocalteu evaluation. It may be due to the present of some phenolic compound such as methyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate according to the previously report[9]. Therefore, the characterizations by using further technique such as GC-MS

TABLE-1
TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF CRUDE EXTRACTS OF *J. curcas* FRUIT

Crude methanolic extract (g/g of sample)	Total phenolic content (mg GAE/g extract)	Antioxidant activity	
		Phen assay (µmol Fe/g extract)	DPPH assay as IC_{50} (mg/mL)
0.3004 ± 0.50	7.04 ± 0.10	270.98 ± 0.59	14.09 ± 0.05

or HPLC-MS may be required for identify the type of interested bioactive compounds. As phen assay mechanism, Oke *et al.*[20], described that the antioxidant activity of phenolic compounds were mainly due to their redox properties as reducing agent. According to 2,2-diphenyl-1-picryhydrazyl method, DPPH$^{\bullet}$ is a stable radical with a maximum absorbance at 517 nm. Its reaction with an antioxidant (AH) compound can be demonstrated by following reaction[21].

$$DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$$

The ability of an antioxidant component in the extracts to scavenge 2,2-diphenyl-1-picryhydrazyl radical was evaluated on the basis of their IC_{50} value, defined as the concentration of sample to decrease the absorbance at 517 nm of DPPH$^{\bullet}$ solution to half of its initial value. The IC_{50} value was obtained by using a calibration curve of the plotting percent inhibition values calculated by eqn. 1 as a function of concentration of the extracts (Fig. 4). The results showed that the crude methanolic extracts gave IC_{50} at 14.09 ± 0.05 mg/mL, as shown in Table-1.

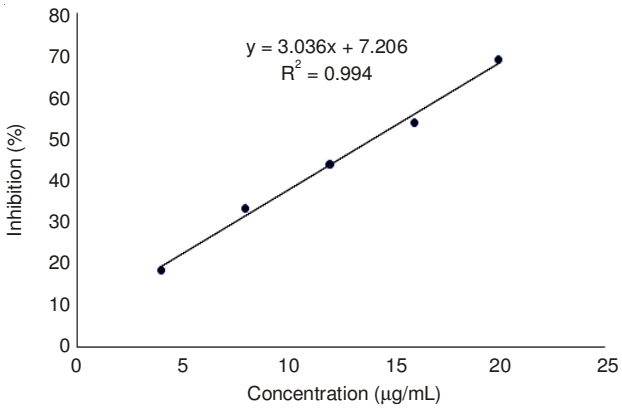


Fig. 4. % Inhibition of 2,2-diphenyl-1-picryhydrazyl assay

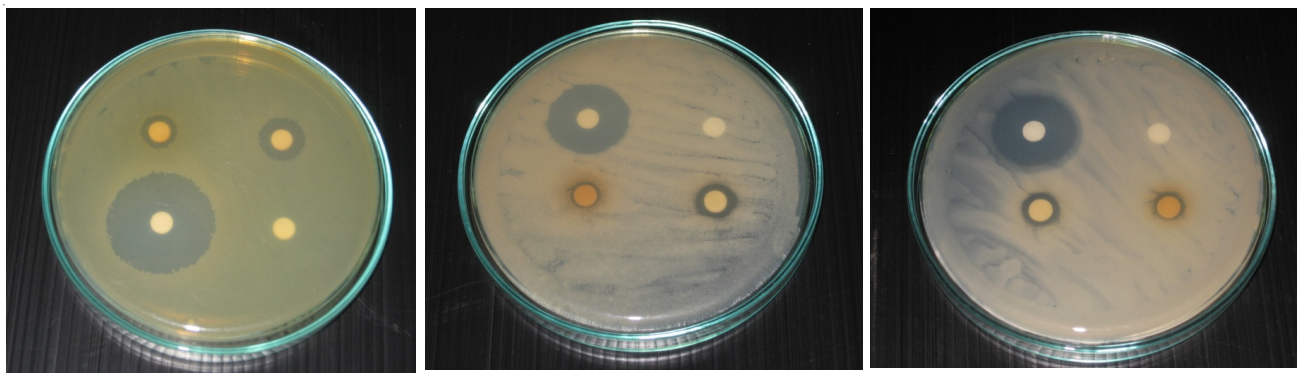
The lower IC_{50} value indicates a stronger scavenging activity of the extracts while the higher IC_{50} value indicates a lower scavenging activity of the extract. The results showed that the methanolic extracts from *J. curcas* fruit had a higher both total phenolic content and antioxidant activity than *J. curcas* seed oil in previous work[9]. Consequently, these results suggested that J. fruit has highly potential as effective natural antioxidant sources.

Antimicrobial activity of the extracts: The antimicrobial activity of the crude methanolic extracts against seven plant pathogenic bacteria were evaluated the potencies by observing inhibition zone and MIC value. The results of antimicrobial activities of the extracts are shown in Table-2. It was found that all extracts exhibited the potencies of antimicrobial activity against six bacterial strains except *P. syringae*. The crude methanolic extracts showed potential for antimicrobial activity against six bacterial strains 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 inhibition zone of crude extracts against some tests bacteria were shown in Fig. 5.

TABLE-2
ANTIBACTERIAL ACTIVITY OF CRUDE METHANOLIC EXTRACTS FROM *J. curcas* FRUIT

Plant pathogenic bacteria	Antimicrobial activity	
	Inhibition zone (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

The results showed that the crude methanolic extracts showed lower antimicrobial activities than that of gentamycin. They exhibited antimicrobial activity with varying degrees of growth inhibition zone against the bacterial strains. As the reported Scorzoniet *et al.*[22], the extract with MIC less than 75 µg/mL was considered to have strong antimicrobial activity, MIC 75-150 µg/mL, the antimicrobial activity was moderate and MIC over 250 µg/mL the extract was considered inactive. Accordingly, the crude methanolic extract in this study possessed moderate antimicrobial activity against *P. putida*, *X. campestris*, *X. campestris* pv. *glycines*, *X. campestris* pv. *vesicatoria*. Antimicrobial activities may depend on the active compounds such as phenolic compound according to previous report[10]. As the studied of Tongpoothorn *et al.*[9] which reported that the identified compounds such as 2,4-di-*tert*-butylphenol, 3-(3, 5-di-*tert*-butyl-4-hydroxyphenyl) propionate and linoleic acid may cause the extracts from *J. curcas* seed oil exhibited



(a) (b) (c)

Fig. 5. Disc diffusion inhibition zone of (a) *Pseudomonas putida*, (b) *Pseudomonas syringae* pv. *sesami*, (c) *Xanthomonas campestris* pv. *glycines*

antimicrobial activities[9]. However, it was found that the crude methanolic extract from *J. curcas* fruit had a higher both total phenolic content and antioxidant activity than *J. curcas* seed oil in previous work. Therefore, the compound present in the crude methanolic extract from *J. curcas* fruit can be introduced as potential candidate for natural antimicrobial sources.

Conclusion

The results obtained from the present study showed that the extracts 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 extracts exhibited good antioxidant activities determined by using the Phen and 2,2-diphenyl-1-picryhydrazyl methods. Therefore, the compounds present in the crude methanolic extracts can be introduced as potential candidate for natural bioactive sources. The findings of this study by several techniques are useful to further research to identify, isolate and characterize the specific compounds which is responsible for higher bioactive activities.

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