Available online at www.elixirpublishers.com (Elixir International Journal)

Organic Chemistry

Elixir Org. Chem. 67 (2014) 21438-21441

ISSN: 2229-712X

Phytochemical screening and antibacterial activity of crude extracts from *Jatropha curcas*, Linn against histamine-forming bacteria

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ARTICLE INFO

Article history:

Received: 15 December 2013;

Received in revised form:

22 January 2014;

Accepted: 3 February 2014;

Keywords

Phytochemical,

Antibacterial,

Jatropha curcas,

Histamine forming-bacteria.

ABSTRACT

This study aims to analyze the phytochemical screening and antibacterial activity of leaves extract of *Jatropha curcas* against histamine forming bacteria, and determine of minimum inhibitory concentration. The extraction process is done by maceration method using hexane, chloroform, ethyl acetate and methanol. The extract was obtained continued by antibacterial activity test against histamine-forming bacteria. Concentration all extract of *J. curcas* is 1%, 10%, 20%, and 30% (w / v). Positive control using synthetic antibiotic ampicillin and negative controls used dimethyl sulfoxide (DMSO). The results of phytochemical screening showed that ethyl acetate and methanol extracts containing 5 secondary metabolites, while the hexane and chloroform containing only 2 secondary metabolites. Antibacterial activity test showed that the negative control and positive control do not provide inhibition against test bacteria. All types of extracts with concentrations of 1% to 30% (w / v) gave inhibition against test bacteria. The diameter of the inhibition of *K. pneumoniae* and *C. perfringens* for all jatropha leaf extract at a concentration of 1% (w / v) did not show any significant difference to the concentration of 10%. Lowest MIC (0.10% w / v) there at the ethyl acetate extract (E3) and methanol (E4) for the bacteria *C. perfringens.*

Introduction

Jatropha curcas, a tropical plant belonging to the family of *Euphorbiaceae* is cultivated mainly as a hedge in many Latin American, Asian and African countries. They are use as a traditional medicine. *Jatropa curcas* leaves are often used as a medicine for skin infections, the seed is used for constipation, treating cervical cancer, and fungal infections[1]. In some countries, *J. curcas* used as a drug, such as malaria medicine in Mali[2] and in Africa as a drug haemostatik[3], skin infections, diarhea, and several other diseases caused by microorganisms[4,5]. Beside to traditional medicine, *J. curcas* used to histamine poisoning prevention. Fishing communities in Maluku, Indonesia, often adding a few leaves of *J. curcas* on the boiling process of the frigate tuna (*Auxis thazard thazard*, L) that its quality has begun to decline. The purpose of added the leaves of Jatropha is to prevent histamine poisoning when the fish is consumed. Frigate tuna, included in the scombridae family. Some types of Scombroid, contains the amino acid histidine is high enough in the flesh. Histidine can be converted into histamine by the enzyme histidine decarboxylase[6]. Formation of histamine in fish may occur because the enzyme histidine decarboxylase (HDC), which exists naturally in the flesh of fish or HDC enzyme produced by bacteria. Various types of bacteria that can produce the enzyme HDC included in the family Enterobacteriaceae and Bacillaceae[7]. The types of histamine-forming bacteria found in marine fish is *Hafnia alvei*, *Klebsiella pneumoniae*, *Escherichia coli*, *Clostridium perfringens*, *Lactobacillus* sp., *Enterobacter aerogenes*, and *Proteus morganii*[8]. Previous studies have reported that *J. curcas* exhibits antimicrobial activity[9,10,11]. The crude stem extracts of *J. curcas* to inhibit the growth of bacteria family Enterobacteriaceae like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*[12]. This research to tested the antibacterial activity of leaves extract of Jatropha curcas against histamine forming bacteria are included in the family Enterobacteriaceae.

Material and Methods

Material

Jatropha curcas leaves were collected from Waitatiri village, Ambon, Maluku Province, Indonesia, where the plant grows under natural condition. *J. curcas* leaves are used in this study is that older leaves (dark green). The organic solvent used is hexane, chloroform, ethyl acetate and methanol, distilled water, filter paper, nutrient agar (NA), nutrient broth (NB) and histamine forming bacteria culture namely, *Klebsiella pneumonia* and *Clostridium perfringens* were obtain from the Microbiology laboratory, Faculty of Medicine, University of Indonesia. The microorganisms were maintained at 4°C on Nutrient Agar slant in the Microbiology laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia and fresh subcultures were made before use.

Equipment

The equipment used was an analytical balance, bottles, funnel, rotary evaporator vacuum, petri dish, beaker glass, paper, cotton, needle ose, incubators, electric cooker, autoclave, Bunsen, micro pipette, calipers and a set of glassware.

Research Methods

Extraction

The powdered of leaves of *J. curcas* macerated with hexane, then filtered. The residue was macerated again with different solvents, namely chloroform, ethyl acetate and methanol. Separately, powder of leaves of *J. curcas* specifically macerated with methanol. The length of time for each maceration solvent is 3 x 24 hours. Furthermore, each filtrate

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evaporated with a rotary evaporator vacuum. Thus, the entire amount of the extract obtained are 5 types.

Phytochemical Screening

The extracts were subjected to phytochemical tests for plant secondary metabolites, alkaloids, flavonoids, phenolic, saponins, tannins, terpenoids and steroids[13] with little modification.

Antibacterial Activity Test

Antibacterial activity test of *J. curcas* extract using the streak plate method [14] with procedures: solid medium is heated until melted, then cooled to a temperature of ± 40 ^{o}C. After that, poured in a sterile petri dish is then allowed to become solid. Take a bacterial culture with ose needle, then scratch at the surface of the agar medium until evenly. Paper discs dripped with 20 µL of the four extracts. The fourth extract concentration was 1%, 10%, 20%, and 30% (w / v). Positive control using synthetic antibiotic ampicillin and negative controls using dimethyl sulfoxide (DMSO). Paper discs containing extracts and controls placed on the surface of an agar medium in a petri dish, then covered petri disah. Furthermore petri dishes were incubated at 37 ^{o}C for 18-24 hours. The diameter of the inhibition zone formed was measured using calipers[15].

Determination of Minimum Inhibitory Concentration (MIC) of Extract

Jatropha curcas extract that provides an inhibitory effect on the *K. pneumoniae* and *C. perfringens* followed by determination of minimum inhibitory concentration using the streak plate method (the same as the determination of antibacterial activity). Variations in the concentration of *J. curcas* leaves extract ranging from 0.10% (w / v) to 0.75% (w / v). The diameter of the inhibition zone formed was measured using calipers[15].

Time and Research Location

This study was conducted from June to October 2013. Extraction process, phytochemical screening, test antibacterial activity, and determination of MIC leaves extract *J. curcas* performed at the laboratory of Phytochemistry and Microbiology, Faculty of Pharmacy, University of Hasanuddin, Makassar, Indonesia.

Result and Discussion

The extract obtained from extraction process of Jatropha curcas leaves shaped viscous liquid (except chloroform, shaped solid) and colored green-black. The green color in the extracts due to the presence of chlorophyll which its extracted from the leaves of Jatropha. The compounds that can be extracted from the material includes a plurality of primary metabolites such as lipids, proteins, starch, pectin and chlorophyll / pigments and secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, steroids and tannins [16].

Phytochemical screening

Phytochemical screening was conducted to determine the types of secondary metabolites contained in the leaves of Jatropha. This analysis is very useful to determine the main classes of active compounds from the leaves of Jatropha curcas which has antibacterial activity. Results of phytochemical screening of the leaf extract of Jatropha can be seen in Table 1.

Table 1 shows that the highest content of secondary metabolites present in the ethyl acetate extract (E3) and methanol (E4), followed by methanol extract (E5), hexane (E1) and chloroform (E2). This means that secondary metabolites contained in the leaves of Jatropha largely are compound of semipolar and polar. Alkaloids are organic compounds containing basic nitrogen atom in the structure as part of a cyclic system. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *J. curcas*. These secondary metabolites exert antimicrobial activity through different mechanisms. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines[17]. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications[18]. Flavonoids, another constituent of *J. curcas* extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties[19]. One class of compounds has potential antimicrobial terpenoids are triterpenoids. Triterpenoids including compounds that are the active components in the herbs that have been used for skin disorders, serve as an antifungal, insecticidal, antibacterial or virus[20]. Tannins have been found to form irreversible complexes with prolinerich protein[21] resulting in the inhibition of cell protein synthesis. Tannins are known to react with proteins to provide the typical tannins effect which is important for the treatment of inflamed or ulcerated tissues[22]. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery[23]. Saponin was found to be present in *J. curcas* extracts and has supported the inhibitory effect on inflamed cells[24]. Steroidal compounds present in *J. curcas* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones[25]. Phytochemical screening of the plant Jatropha has been done by several researchers and showed the same results as found in this study. Methanol extract of leaves of Jatropha contains compounds alkaloids, flavonoids, terpenoids, saponins, tannins and steroids[26]. Jatropha leaves contain tannin, alkaloids, steroids and saponins[9]. Methanol extract of leaves of Jatropha contains alkaloids, flavonoids, terpenoids, phenols, tannins and steroids, while the chloroform extract contains flavonoids and tannins [27].

Table 1. Phytochemical screening of Jatropha curcas leaves extracts

Secondary metabolites	Extract type				
	Hexane (E1)	Chloroform (E2)	Ethyl Acetat (E3)	Methanol (E4)	Metanol* (E5)
Alkaloids	+	-	+	-	+
Flavonoids	-	-	-	+	-
Phenolic	-	-	+	+	-
Saponins	-	-	+	-	-
Tannins	-	+	+	+	+
Terpenoids	-	-	+	+	-
Steroids	+	+	-	+	+

*= Single maceration

Antibacterial activity

The test results showed that the antibacterial activity of the negative control (DMSO) and positive control (ampicillin) do not provide inhibition against the histamine forming bacteria (*K. pneumonia* and *C. perfringens*). Positive controls that do not provide inhibition against test bacteria showed that bacteria are already resistant to the synthetic antibiotic ampicillin were used in this study. Prior to analysis of variance, all data diameter of the inhibition of both histamine-forming bacteria were conducted test normality of the data. Based on the test results of the normality of data, it turns out the diameter of the inhibition of both histamine-forming bacteria are not spread follow a normal distribution, therefore, the data is transformed $\sqrt{Y}$.

Inhibition Diameter of *Klebsiella pneumoniae*

Analysis of variance showed that treatment of type extract (E) and the concentration of extract (K) was highly significant different (P <0.01) on the diameter of the inhibition of *K. pneumoniae*, while the interaction between the two treatments (E * K) significant different (P> 0.05). HSD test (Table 2) showed that the highest inhibition diameter of *K. pneumoniae* present on the interaction of treatment the methanol extract with concentration of 30% (E5K30) ie 3.87 or 14.98 mm. Based on HSD test, E5K30 treatment was not significantly different to the treatment E5K20, E5K10, E4K30, E2K30 and E2K20, but in contrast to other treatments. In addition, the diameter of the inhibition of *K. pneumoniae* for all jatropha leaf extract at a concentration of 1% (w / v) did not show any significant difference to the concentration of 10%.

Table 2. HSD test of diameter of inhibition *K. pneumoniae* on the treatment interaction of type extract (E) and the concentration of extract (C) from leaves of *Jatropha curcas*

Treatment interaction	**Average**
E1K1	2,56 e
E1K10	2,76 e
E1K20	2,85 cde
E1K30	2,93 cde
E2K1	2,84 cde
E2K10	3,24 bcd
E2K20	3,85 a
E2K30	3,49 ab
E3K1	2,85 cde
E3K10	2,86 cde
E3K20	2,90 cde
E3K30	2,90 cde
E4K1	2,79 de
E4K10	2,79 de
E4K20	2,89 cde
E4K30	2,86 cde
E5K1	2,87 cde
E5K10	3,29 bc
E5K20	3,77 a
E5K30	3,64 ab
$HSD\alpha_{0.05}$ = 0.45	

Note : Numbers followed by the same letter in the same column, are not significantly different at the level test HSDα0.05

Many factors and circumstances that may affect the antibacterial working, among others, the concentration of antibacterial agents, the number of bacteria, species of bacteria, the organic matter, temperature and pH of the environment[28]. Inhibition by antimicrobial compounds in general can be caused by disturbances in the component of the cell and cytoplasm membrane, inhibition of protein synthesis and interference with the function of the genetic material[29].

Inhibition Diameter of *Clostridium perfringens*

Analysis of variance showed that treatment of type extract (E), the concentration of extract (K) and the interaction between the two treatments (E * K) was highly significant different (P <0.01) on the diameter of the inhibition of *C. perfringens.* HSD test (Table 3) showed that the highest inhibition diameter of *C. perfringens* present on the interaction of treatment the methanol extract with concentration of 30% (E4K30) ie 4.02 or 16.16 mm. Based on HSD test, E4K30 treatment was not significantly different to the treatment E4K20, E5K20, E5K30 dan E2K30, but in contrast to other treatments. In addition, the diameter of the inhibition of *C. perfringens* for all jatropha leaf extract at a concentration of 1% (w / v) did not show any significant difference to the concentration of 10%.

Resistance of bacteria to antimicrobial compounds closely related to the structure of the cell wall. Clostridium perfringens is a Gram-positive bacteria, whereas K. pneumonia is the Gram-negative bacteria. The cell walls of gram-positive bacteria have only one membrane layer containing peptidoglycan (± 90%) and a thin layer of sour teikoat, whereas gram-negative bacteria contain a peptidoglycan layer about 5-20%. Teikoat acid caused cell surface of gram-positive bacteria are polar and have a negative charge. These trait will affect the rate of penetration of antimicrobial compounds into cells that can eventually lead to cell leakage. Cell wall of Gram-negative bacteria is more complex, having inner and outer membranes. Layer of the outer membrane contains phospholipids, lipopolysaccharides and lipoproteins. This layer is impermeable to large molecules, but small molecules can pass Lipopolysaccharide and peptidoglycan is a sieve for a wide range of molecular sizes, whereas the plasma membrane is impermeable to molecules whose size is much smaller[14].

Table 3. HSD test of diameter of inhibition *C. perfringens* on the treatment interaction of type extract (E) and the concentration of extract (C) from leaves of *Jatropha curcas*

Treatment interaction	**Average**
E1K1	2,81 d
E1K10	2,85 d
E1K20	2,90 cd
E1K30	2,88 cd
E2K1	2,85 d
E2K10	3,10 cd
E2K20	3,31 bcd
E2K30	3,45 abc
E3K1	2,82 d
E3K10	2,80 d
E3K20	2,85 d
E3K30	2,90 cd
E4K1	3,05 cd
E4K10	3,32 bcd
E4K20	3,79 ab
E4K30	4,02 a
E5K1	2,98 cd
E5K10	3,28 bcd
E5K20	3,78 ab
E5K30	3,71 ab
BNJ 0,05 = 0,58	

Note : Numbers followed by the same letter in the same column, are not significantly different at the level test HSDα0.05

Minimum inhibitory concentration of extracts from *J. curcas* leaves

All leaf extract of Jatropha which provide inhibition against test bacteria was continued by determination of minimum inhibitory concentration (MIC). In this study, the MIC is expressed as the lowest concentration of leaf extract of Jatropha which provide inhibition against both types of histamine-forming bacteria. MIC leaf extract of Jatropha vary depending on the type of test bacteria. Lowest MIC (0.10% w / v) there at at the ethyl acetate extract (E3) and methanol (E4) for the bacteria *C. perfringens*, whereas most of MIC of the leaf extract of Jatropha were at a concentration of 0.50% (w / v) and 0.75% (w / v) (Table 4).

Table 4. Minimum inhibitory concentration of five extracts from *J. curcas* leaves

Crude Extract	**MIC (% w/v)**	
	K. pneumoniae	***C. perfringens***
Hexane (E1)	0.75	0.75
Chloroform (E2)	0.75	0.50
Ethyl Acetat (E3)	0.50	0.10
Methanol (E4)	0.75	0.10
Methanol* (E5)	0.50	0.50

* = Single extraction

These results indicate that *C. perfringens* is more sensitive to the ethyl acetate extract (E3) and methanol (E4) compared with *K. pneumonie*. When associated with phytochemical screening results (Table 1), ethyl acetate extract contains alkaloids, phenolics, saponins, tannins and terpenoids, while the methanol extract containing flavonoids, phenolics, tannins, terpenoids and steroids. Several studies have reported that these secondary metabolites have antimicrobial activity. In addition, *C. perfringens* is a Gram-posifive, where gram-positive bacteria are generally more sensitive to antibacterial compounds than Gram-negative bacteria. This is due to differences in cell wall structure.

Conclusion

Based on the results of this study, it could be concluded that the highest content of secondary metabolites present in the ethyl acetate extract (E3) and methanol (E4), followed by methanol extract (E5), hexane (E1) and chloroform (E2). All of *Jatropha curcas* extracts have antibacterial activity against histamine-forming bacteria, namely *Klebsiella pneumoniae* and *Clostridium perfringens*. Lowest MIC (0.10% w / v) there at at the ethyl acetate extract (E3) and methanol (E4) for the bacteria *C. perfringens*.

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