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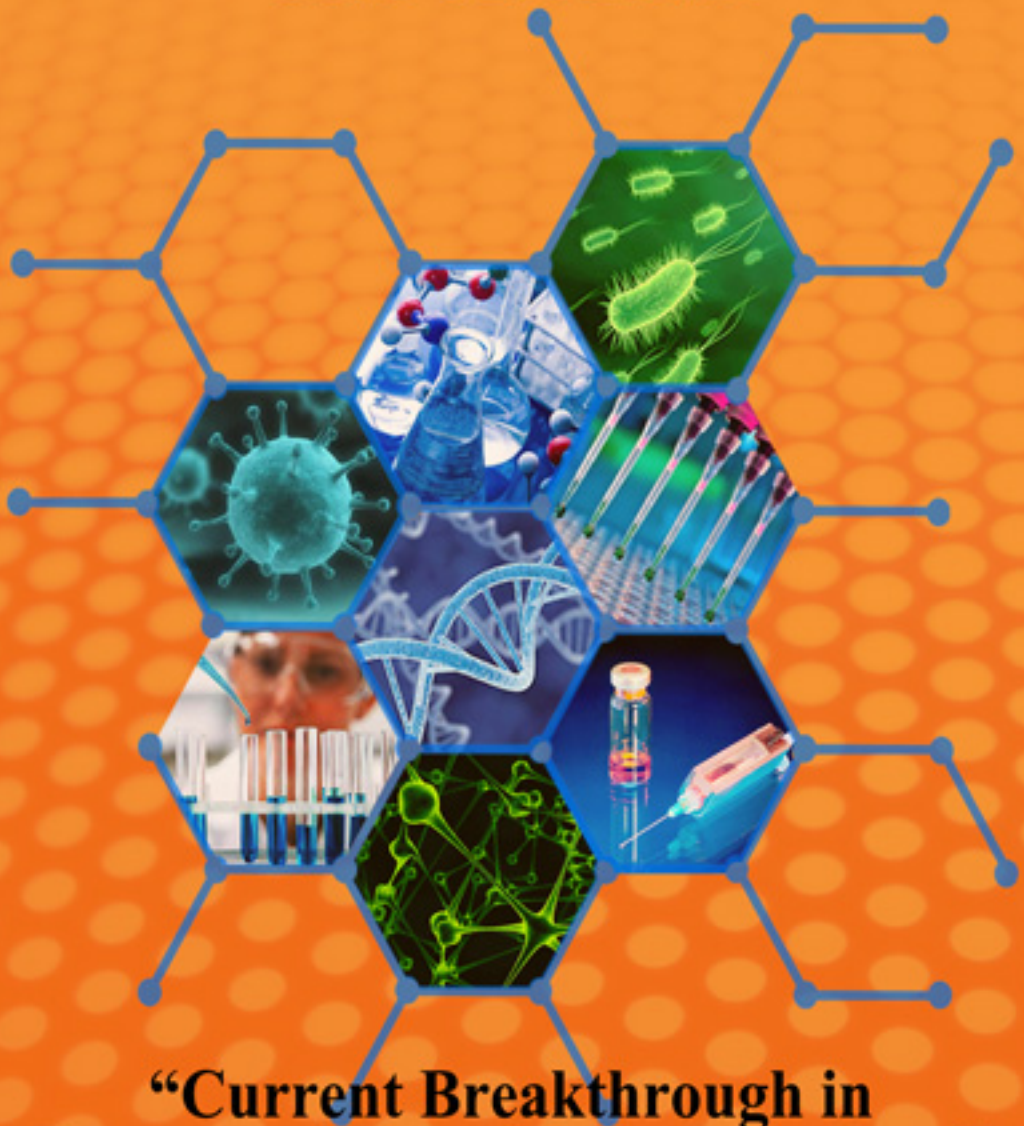

"Current Breakthrough in Pharmacy Materials and Analyses"

Auditorium of Muhammad Djazman Al Kindi
Muhammadiyah University of Surakarta, Solo, Central Java, Indonesia

Published by :

2015

Surat Keterangan
Pengalihan Ijin Publikasi Mandiri

Kami, panitia pelaksana kegiatan ***International Conference ICB Pharma*** pada tanggal 10 Januari 2015 di Auditorium Mochammad Djazman UMS Surakarta, memberikan ijin Pengalihan Publikasi Mandiri secara online artikel dari:

Nama Penulis: Yeni Cristiana and Ratna Yuliani
Asal Institusi : Fakultas Farmasi Universitas Muhammadiyah Surakarta
Judul Artikel : ***Comparison of Antibacterial Activity of Ethanolic Extract of Seed and Stem Papaya*** **(Carica papaya, L)** ***against*** **Shigella dysenteriae** ***and*** **Streptococcus pyogenes.**

untuk dipublikasi secara Mandiri oleh yang bersangkutan. Artikel tersebut telah didokumentasikan dalam *Proceeding* ***International Conference ICB Pharma*** dengan nomor ISBN 978-602-70429-9-5 yang diterbitkan oleh Muhammadiyah University Press. Demikian surat ini dibuat agar dapat dipergunakan sebagaimananya mestinya.

Jakarta, 27 Februari 2015

Panitia Pelaksana

ICB Pharma 2015
Faculty of Pharmacy
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Anita Sukmawati, Ph.D., Apt..

Proceeding International Conference
"Current Breakthrough in Pharmacy Materials and Analyses" / Nurwaini *et al.,* (ed)
Surakarta : Muhammadiyah University Press, 2015

xxi, 153 pages
ISBN: 978-602-70429-9-5

Pharmacy

Published by:
Muhammadiyah University Press
Universitas Muhammadiyah Surakarta
Jl. A. Yani Pabelan Tromol Pos I Kartasura Surakarta 57102
Telp. (+62 271) 717417-172, E-mail: muppress@yahoo.com

PREFACE

It's my great pleasure to welcome you to the 1st International Current Breakthrough (ICB)-Pharma Symposium 2015 in Solo Indonesia which will be held on 10 January, 2015 under the auspices of Universitas Muhammadiyah Surakarta.

ICB-Pharma 2015 will feature a theme of "Current Breakthrough in Pharmacy Material and Analyses" and will consist of morning and afternoon sessions. There will be plenary lectures and session lectures given by several invited speakers from Japan, South Korea, Taiwan as well as from Indonesia, and also selected oral presentations of the submitted papers. The poster session from various fields of pharmaceutical sciences will take place nearby.

The ICB-Pharma will be directed for a tradition and in the future will be nurtured as a well-known scientific symposium in disseminating the breakthrough and novel technology in pharmacy materials. This purpose will be able to achieve by encouragement of national and international academic institution partners and supports from the industrial partners, Indonesian Pharmacist Association (Ikatan Apoteker Indonesia, IAI), colleagues and from the organizing committee. Moreover, I do hope and believe that, the 1st ICB-Pharma will offer great opportunities for the scientists to meet and discuss recent topics in the field of material and pharmaceutical science and bring the academics, health professionals and industries together for sharing their experiences to solve current problems and challenges in practice.

Warm regards,

Anita Sukmawati, PhD
Chair of ICB-Pharma Symposium

ISBN : 978-602-70429-9-5

PREFACE

This conference is held to disseminate current methods which provide advanced materials and methods in pharmacy. This a good media for those who are engaged in academic, industrial, regulatory fields to conduct social interactions, to share their findings, to communicate bottle neck surrogates, and to seek the possibilities for collaborations. Since it is our initial moment, we are quite humble to recognize our weakness in running all agendas. Hence, from bottom of our heart we ask apologizes from all participants for any service, lack facilities, low response, etc.. However, we must be persistent and ensuring our positive contribution toward scientific society especially at the attempt to develop capacity building of pharmaceutical sciences in Indonesia. Thus, we will run ICB Pharmacy II in 2015/2016.

To all participants, I wish an inspiring moment in Solo city, a city where was born a national leader and city of heritage !

Azis Saifudin, PhD

Dean of Faculty of Pharmacy
Universitas Muhammadiyah Surakarta

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ISBN : 978-602-70429-9-5

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ACKNOWLEDGEMENT

The authors are grateful for financial support by Lembaga Pengelola Dana Pendidikan (LPDP) Republic of Indonesia.

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COMPARISON OF ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF SEED AND STEM PAPAYA (*Carica papaya* L.) AGAINST *Shigella dysenteriae* AND *Streptococcus pyogenes*

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Abstract

Generally, seed and stem of papaya (*Carica papaya* L.) are not used. In a previous study, seed and stem of papaya inhibit growth of *Escherichia coli* and *Staphylococcus aureus*. The aim of this study was to compare antibacterial activity of ethanolic extract of papaya seed to stem extract against *Shigella dysenteriae* and *Streptococcus pyogenes*. Papaya seed and stem were extracted using 70% ethanol by maceration method. The antibacterial activity tests were carried out using Kirby-Bauer disk diffusion method. Chemical compounds in the extracts were identified using thin layer chromatography (TLC) with silica GF_{254} as stationary phase and a mobile phase of ethyl acetate : methanol : water (100 : 13 : 17)v/v for papaya seed extract and methanol:chloroform (8:2)v/v for papaya stem extract. The results showed that ethanolic extract of papaya seed and stem have same antibacterial activity against *Shigella dysenteriae* and *Streptococcus pyogenes*. Based on TLC analysis, papaya seed contain alkaloid, steroid, and tannin, while papaya stem contain tannin.

Keywords: papaya (*Carica papaya* L.), antibacterial, *Shigella dysenteriae, Streptococcus pyogenes.*

INTRODUCTION

Papaya (*Carica papaya* L.) has many useful parts but not all of them are used. Seed and stem are usually discarded, though both of them contain a variety of compounds. Papaya seed contains alkaloid such as carpain, and glycosides such as glucotropaelin and benzyl-isothiocyanate (Nayak, 2012). Papaya stem contains alkaloid, tannin, saponin, steroid (Stephen et al., 2013) and anthraquinone (Setiawan, 2009).

Methanolic extract of papaya stem decreases blood sugar level in mice significantly (Saidu and Nweri, 2013) and ethanolic extract of papaya seed has antifertility activity (Hamman et al., 2011). In addition, papaya seed and stem have antibacterial activity. Ethanolic extract of papaya seed have antibacterial activity against *Staphylococcus aureus*

and *Escherichia coli* with inhibition zone diameter of 13 and 17 mm, respectively (Okoye, 2011). Ethanolic extract of papaya stem with concentration of 0.5 g/mL inhibit *Escherichia coli* with inhibition zone diameter of 20 mm (Khan et al., 2014).

Active compounds in the seed and stem of papaya may solve the problem of resistance in the treatment of infections. The presence of infection in the body causes a variety of diseases (Tambayong, 2000). *Shigella dysenteriae* is a Gram-negative bacterium of genus *Shigella* that commonly causes gastrointestinal infection (Radji, 2009). *Shigella* cause bloody mucoid diarrhea that is transmitted directly through food and drinking water (Sears et al., 2011). Diarrhea caused death of 5 million people each year, especially children (Shulman et al., 1994). *Streptococcus pyogenes* is a Gram-positive bacterium of genus *Streptococcus* that can spread infection by releasing a toxin. The bacteria cause skin, circulatory and respiratory system infections (Radji, 2009). This study was carried out to compare antibacterial activity of ethanolic extract of seed to stem extract of papaya (*Carica papaya* L.) against *Shigella dysenteriae* and *Streptococcus pyogenes.*

MATERIALS AND METHODS

Materials

Papaya seed and stem were obtained from Ringinsari, Randusari Village, District Teras, Boyolali, *Shigella dysenteriae* and *Streptococcus pyogenes* were obtained from Center of Health Laboratory of Yogyakarta, 70% ethanol, Mueller Hinton (MH) medium, Brain Heart Infussion (BHI), tetracycline, erythromycin, silica gel GF_{254}, chloroform, methanol, ethyl acetate, distilled water, $FeCl_3$, Dragendorff, ethanolic-KOH, Liebermann-Burchard (LB), and ammonia vapour.

Methods

Preparation of extract

White papaya seeds were taken from unripe papaya fruits which were about 2 to 5 months old. Papaya stem were taken from papaya trees that bear fruit. Briefly, 310 g of seed powder was extracted using 2.8 L 70% ethanol and 450 g of stem powder was extracted using 3.5 L of 70% ethanol in different maceration jar for 3-5 days and stirred manually every day. Papaya seed and stem extracts were filtered and concentrated using rotary evaporator and water bath.

Preparation of Inocula

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Three or five pure colonies of *Shigella dysenteriae* and *Streptococcus pyogenes* were touched with a sterile wire loop, suspended in 3 mL of Brain Heart Infussion and shook until reached the turbidity of McFarland's standard.

Antibacterial activity test

Papaya seed and stem extracts were dissolved in 1.5 mL 70% ethanol to give a series concentration of 50%, 25%, 12.5%, and 6.25%. 20 µL of the extracts with concentrations of 50%, 25%, 12.5%, and 6.25% were loaded on sterile discs to give concentration of 10000 µg, 5000 µg, 2500 µg, and 1250 µg, respectively. Papaya seed and stem extracts were tested for antibacterial activity using the Kirby-Bauer disc diffusion method. Mueller Hinton agar plates were inoculated with 300 µL of *Shigella dysenteriae* ($1.5x10^8$ CFU/mL) and 200 µL *Streptococcus pyogenes* ($1.5x10^8$ CFU/mL). After 20 min, 4 extract-loaded discs, 1 antibiotic disc (30 µg tetracycline disc as positive control for *Shigella dysenteriae* and 30 µg erythromycin disc for *Streptococcus pyogenes),* and 1 70% ethanol-loaded disc (as a negative control) were transferred onto the agar surface of each plate using sterile forceps. The plates were then incubated at 37C for 24 hours. The activity of the papaya seed and stem extract as antibacterial against *Shigella dysenteriae* and *Streptococcus pyogenes* was determined by measuring the diameter of inhibition zones.

Thin Layer Chromatography (TLC)

Chemical compounds in the papaya seed and stem extracts were identified using thin layer chromatography (TLC) with silica GF_{254} as stationary phase and a mobile phase of ethyl acetate:methanol:water (100:13:17) v/v for papaya seed extract and methanol:chloroform (8:2)v/v for papaya stem extract. 20 uL of extract solution with concentration of 50% were spotted on silica GF_{254} plates that had been activated by heating at 110C for 1 hour. When the sample has dried up, elution was carried out and the plate were removed when it reached the upper part of the silica plate. The spots on the silica plate were detected using visible light, UV 254 nm, 366 nm, ammonia vapour, and spray reagents such as $FeCl_3$ Dragendorff, ethanolic- KOH, Liebermann-Burchard (LB).

RESULTS AND DISCUSSION

Antibacterial activity test

The antibacterial activity of ethanolic extract of papaya seed and stem against *Shigella dysenteriae* were shown in Table 1 and Figure 1.

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Table 1. Antibacterial activity of ethanolic extract of papaya seed and stem against *Shigella dysenteriae* and *Streptococcus pyogenes*

Samples		Diameter of inhibition zones (mm)	
		Shigella dysenteriae	*Streptococcus pyogenes*
Papaya seed extract	10000 µg	7,66±0,57*	7±0*
	5000 µg	7,33±0,57*	7±0*
	2500 µg	6±0	6±0
	1250 µg	6±0	6±0
70% ethanol	14000 µg	6±0	6±0
Tetracycline	30 µg	18,33±0,57	-
Erythromycin	30 µg	-	18,33±1,52
Papaya stem extract	10000 µg	8±0*	7,33±0,57*
	5000 µg	7±0*	7±0*
	2500 µg	6,33±0,57*	6±0
	1250 µg	6±0	6±0
70% ethanol	14000 µg	6±0	6±0
Tetracycline	30 µg	16±1,73	-
Erythromycin	30 µg	-	16,66±0,57

*irradical zone

Diameter of inhibition zone include disc diameter (6 mm)

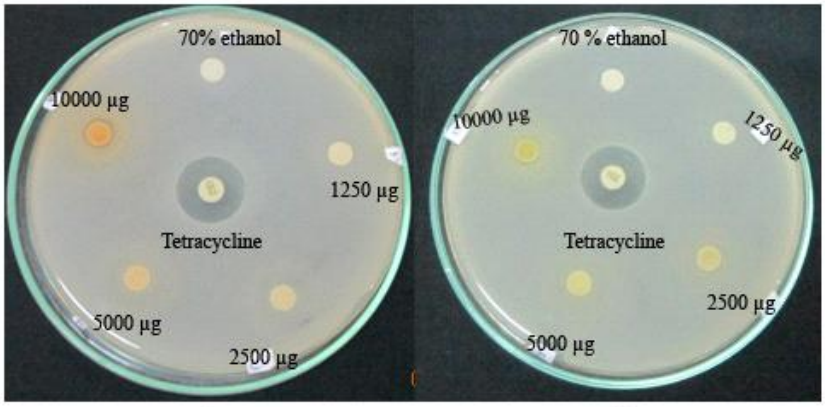

Figure 1. Antibacterial activity of ethanolic extract of papaya seed (A) and stem (B) against *Shigella dysenteriae*

Ethanolic extract of papaya seed at concentration of 10000 µg and 5000 µg have antibacterial activity against *Shigella dysenteriae* that were showed as irradical zone with diameter of 7,66±0,57 and 7,33±0,57 mm, respectively. Papaya seed extract at concentration of 2500 µg and 1250 µg gave no clear zone around the disc. It showed that the concentration did not inhibit the growth of *Shigella dysenteriae*. Ethanolic extract of

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papaya stem extract at concentration of 10000 µg, 5000 µg and 2500 µg have antibacterial activity against *Shigella dysenteriae* with inhibition zone diameter of 8±0, 7±0 and 6,33±0,57 mm, respectively. Papaya stem extract at concentration of 1250 µg have no antibacterial activity. Ethanolic extract of papaya seed and stem have same antibacterial activity against *Shigella dysenteriae.*

The antibacterial activity of ethanolic extract of papaya seed and stem against *Streptococcus pyogenes* were shown in Table 1 and Figure 2.

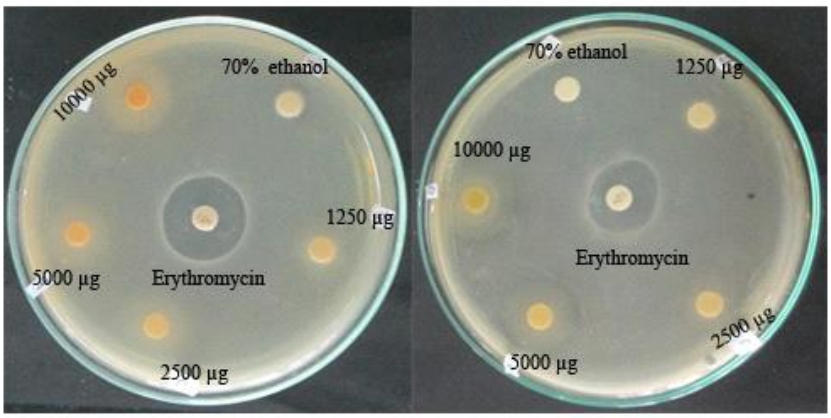

Figure 2. Antibacterial activity of ethanolic extract of papaya seed (A) and stem (B) against *Streptococcus pyogenes*

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Irradical zones with diameter of 7±0 mm were observed around discs containing papaya seed extract with concentration of 10000 µg and 5000 µg. It indicated that the seed extracts have antibacterial activity against *Streptococcus pyogenes.* Papaya seed extract at concentration of 2500 µg and 1250 µg did not have antibacterial activity against *Streptococcus pyogenes.* Papaya stem extract at concentration of 10000 µg and 5000 µg gave inhibition zone with diameter of 7.33±0.57 and 7±0 mm, respectively. Papaya stem extract at concentration of 2500 µg and 1250 µg did not inhibit the growth of *Streptococcus pyogenes.* Ethanolic extract of papaya seed and stem have same antibacterial activity against *Streptococcus pyogenes.* The ethanolic extract of papaya stem have inhibition zone of 28 mm while the papaya seed did not have inhibition zone against *Staphylococcus aureus (*Khan *et al.,* 2012). Based on the results of antibacterial tests against *Streptococcus pyogenes*, papaya stem extract inhibit Gram-positive bacteria better than papaya seed.

Thin Layer Chromatography (TLC)

TLC results showed that the ethanolic extract of papaya seed contains alkaloid, steroid, and tannin. Alkaloid was shown as brownish color with Rf value of 0.08 after it has been sprayed with Dragendorff. Steroid showed blue fluorescence under UV 366 nm with Rf values of 0.16 and 0.95 after being sprayed with LB. Tannin showed black colour with Rf value of 0.26 after it has been sprayed with $FeCl_3$. TLC results showed that the ethanolic extract of papaya stem contains tannin. Tannin shows black colour after $FeCl_3$ sprayed with Rf value of 0.53. In theory, steroid give a blue fluorescence after being sprayed with LB, alkaloids showed red orange yellow background gray, brown/orange brown after being sprayed with Dragendorff, and tannins show blue, red, purple, green, or black after spraying with $FeCl_3$ (Wagner and Bladt, 1996).

CONCLUSION

The ethanolic extracts of papaya seed and stem have same antibacterial activity against *Shigella dysenteriae* and *Streptococcus pyogenes*. The ethanolic extract of papaya seed contains alkaloid, steroid, and tannin, while the ethanolic extract of papaya stem contains tannin.

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ISBN 978-602-70429-9-5