Isolation and Identification of Active Compounds from Papaya Plants and Activities as Antimicrobial

IOP Conf. Series: Materials Science and Engineering **349** (2018) 012007 doi:10.1088/1757-899X/349/1/012007

Isolation and Identification of Active Compounds from Papaya Plants and Activities as Antimicrobial

A T Prasetya[1], S Mursiti[2], S Maryan[3], N K Jati[3]

[1]Doctoral Degree of Postgraduate, Semarang State University, Indonesia, Kampus Kelud-Petompon-Gajah Mungkur Semarang Indonesia 50237
[2]Chemistry Department, Faculty of Mathematics and Natural Science, Semarang State University, Indonesia, Sekaran Gunungpati Semarang Indonesia 50229
[3]Chemistry Student, Chemistry Department, Faculty of Mathematics and Natural Science, Semarang State University, Indonesia, Sekaran Gunungpati Semarang Indonesia 50229

agungchem@mail.unnes.ac.id

Abstract. Extraction and isolation of papaya seeds and leaves (*Carica papaya L*) has been performed using *n*-hexane and ethanol solvents. Further isolation of the extract obtained using ethyl acetate and diethyl ether solvents. The result of the phytochemical test of papaya extract obtained by mixture of an active compound of flavonoids, alkaloids, tannins, steroids, and saponins. Ethyl acetate isolates containing only flavonoids and diethyl ether isolates contain only alkaloids. Extracts and isolates from papaya plants had gram-positive antibacterial activity greater than the gram-negative bacteria, but both did not have antifungal activity. Papaya extracts have greater antibacterial activity than flavonoid isolates and alkaloid isolates. Strong antibacterial inhibitory sequences are extracts of papaya plants, flavonoid isolates, and alkaloid isolates.

1. Introduction

Indonesia is one of the countries that have biodiversity, but few existing plants have been utilized in the field of medicine. Most of these plants are utilized as traditional medicinal ingredients that have proven efficacy derived from generation to generation [1]. There is very little scientific literature that examines the chemical components and composition of the medicinal plants. One of the most widely used plants in the field of traditional medicine namely papaya (*Carica papaya L*), especially on the seeds and leaves [2]. Traditionally papaya plants have been used as an anthelmintic, digestive disorders, diarrhea, skin diseases, male contraceptives, and raw materials for colds [3].

Artanti *et al*., [4] state that a number of flavonoid-containing medicinal plants have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, antiallergic and anticancer activity. According to Sudibyo [5], part of the papaya plant has bioactive and pharmaco-active properties such as secondary metabolite compounds which are often used in the pharmaceutical field. In papaya leaf extract contained *papain* enzyme that has proteolytic and antimicrobial activity, and alkaloids as antibacterial, flavonoids as an anti-inflammatory [6], [7]. In addition, papaya plants also contain several active antioxidant components [8]. Based on research conducted Delphin *et al*., [9] papaya seed extract contains various kinds of secondary metabolite compounds such as alkaloids, flavonoids, saponins, and tannins.

Infectious diseases are the most common types of diseases suffered by the population of developing countries, including Indonesia [10]. Infections are caused by pathogenic microbes such as bacteria, viruses, fungi, protozoans, or some other minority group (microplasma, rickets, and chlamydia) [11]. Pathogenic microbes, such as *Escherichia coli*, can cause acute diarrhea, as well as a major cause of urinary tract infections. Diseases caused by these infections are usually treated with antibiotics [12]. The content of active compounds in plants is usually in small quantities, so it needs to be isolated and concentrated with various methods, one of the extraction. In order for the desired active compound to be extracted, the choice of solvent used is necessary. Selection of suitable solvents is an important factor in the extraction process. The solvent used is a solvent which can filter out most of the secondary metabolites present in the simplified. The most commonly used solvents are alcohols such as methanol and ethanol [13]–[18]. This study aims to determine the active compounds contained in seeds and leaves of papaya and to test its effectiveness as an antimicrobial.

2. Method

Instruments used in this study are rotary vacuum evaporator (*Heidolph*), *Fourier Transform Infrared Spectrometer* (FT-IR from *Perkin Elmer Frontier Series*), and chemicals with grade pro-analyst made by *Merck*. The bacteria cultures *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* fungi obtained from RSUD Dr. Kariadi Semarang city.

Making Simplicia is done by drying the plant part (seeds and leaves) and then mashed. Mixed Simplicia in maceration using *n*-hexane solvent for 3x24 hours. The obtained residue is dried and macerated back with ethanol solvent. The obtained filtrate was concentrated using a rotary vacuum evaporator. Some of the extracts obtained were isolated using ethyl acetate and diethyl ether solvents to concentrate and isolate further active compounds [3], [6]. Extracts and isolates obtained by phytochemical screening included alkaloid, flavonoid, saponin, tannin, steroid, and terpenoid test, and further identification using FT-IR spectrometer. The extracts and the resulting isolates were then tested for antimicrobials using paper disc method with a long incubation of 1x24 hours.

3. Result and Discussion

The research begins with the collection of seeds and leaves of papaya from Sekaran, Gunungpati, Semarang. Once identified, the samples obtained are then processed into a powder of Simplicia. The next step is extraction using *n*-hexane solvent to remove nonpolar compound components such as oil and fat. The extraction followed by ethanol solvent to take polar compounds such as flavonoids, alkaloids, and others. Some of the extracts obtained are then isolated using ethyl acetate and diethyl ether solvents to extract and concentrate desired polar compounds.

The extract of ethanol (seeds and leaves of papaya) obtained tested its chemical content qualitatively. This test is treated to compare the results obtained with the literature search results, as well as to compare with the results of isolation. Based on the literature, papaya leaf ethanol extract contains compounds such as flavonoids, alkaloids, tannins, saponins [9]. The results of phytochemical tests on extracts and isolates as listed in Table 1.

Table 1. Phytochemical test results

No.	Class test	Papaya seeds		Papaya leaf	
		Extract	Isolate	Extract	Isolate
1.	Flavonoid	+	+	+	-
2.	Alkaloid	+	-	+	+
3.	Tanin	+	-	+	-
4.	Saponin	-	-	+	-
5.	Steroids	+	-	+	-
6.	Terpenoid	+	-	-	-

Table 1 shows that the active compounds of both papaya seed extract and papaya leaf extract are almost the same, which contains flavonoids, alkaloids, tannins, and steroids. The difference is, papaya seed extract does not contain saponins and papaya leaf extract does not contain terpenoid. It differs

precisely in the further isolation results, isolation using ethyl acetate solvent will separate the active compound flavonoids (papaya seed isolates), while isolation with diethyl ether solvent will separate the alkaloid active compound (papaya leaf isolate). In this stage, the choice of solvent type becomes very important depending on what active compounds will be taken. After phytochemical testing, the next step is to identify functional groups using FT-IR instruments to strengthen the results obtained from the phytochemical test. More results as shown in Figure 1.

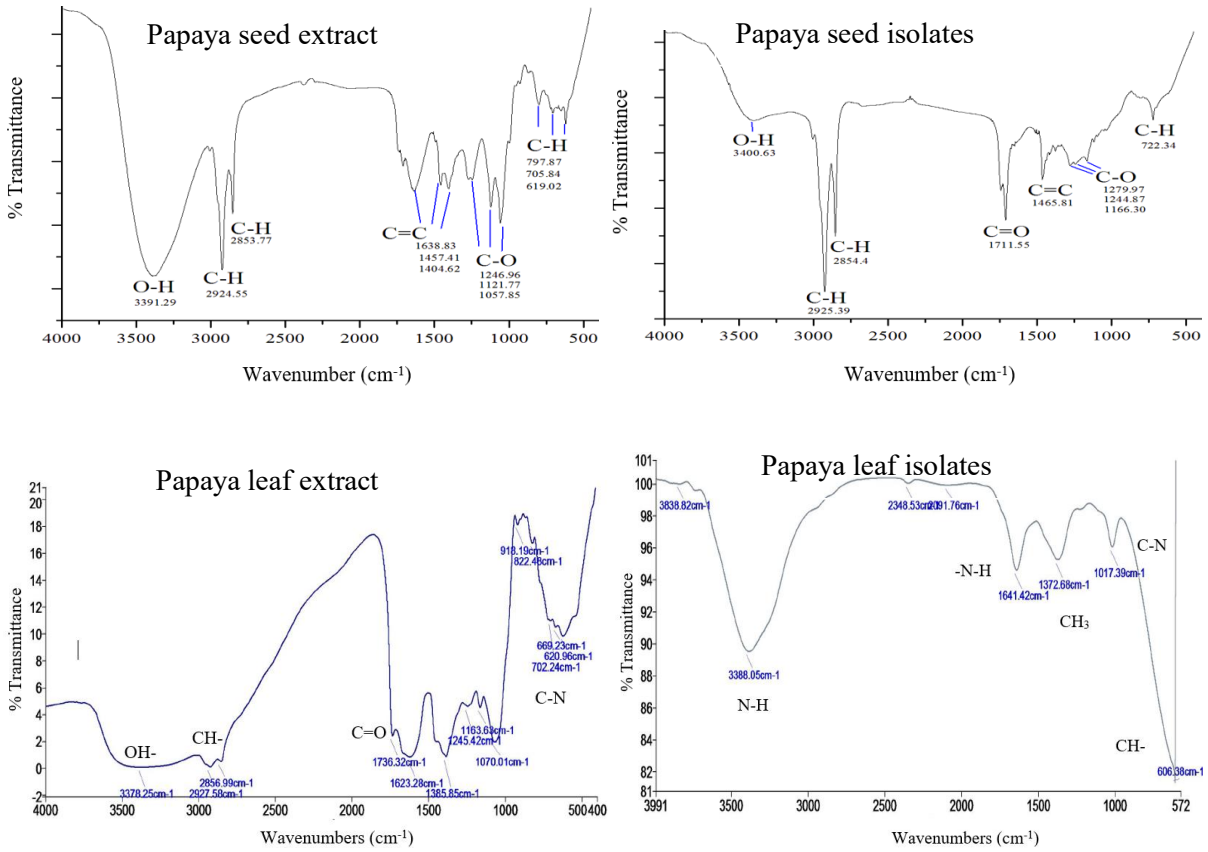

Figure 1. Infrared spectra of extract and isolate from seed and papaya leaf

The result of FT-IR analysis of papaya seed extract showed the presence of -OH group, ie the absorption of widening at wavenumber 3991.29 cm^{-1}. The presence of an aromatic C=C group is indicated by absorption at wavenumbers 1457.41 and 1404.62 cm^{-1}. The presence of vibration C-O is indicated by absorption in the area 1246.96, 1121.77 and 1057.85 cm^{-1}. The presence of an aromatic C-H group out of the field is indicated by the absorption of the fingerprint region in the wavenumber 797.87, 705.84 and 619.02 cm^{-1}. The results of FT-IR analysis of papaya seed isolate showed widespread uptake at 3400.63 cm^{-1} with weak intensity, presumably an uptake of O-H. The sharp aliphatic C-H stretch absorption appears at 2925.39 and 2854.4 cm^{-1} waves. The presence of carbonyl or ketone group (C=O) as one of the common characteristics of flavonoid group compounds is indicated by the absorption of wavenumbers 1711.55 cm^{-1}. The aromatic C=C uptake occurs at the wavenumber 1465.81 cm^{-1}, the vibration of C-O in the wavenumbers 1279.97, 1244.87 and 1166.30 cm^{-1}. Absorption at the wavenumber 722.34 cm^{-1} the presence of aromatic C-H groups out the plane. So it can be concluded that the functional groups contained in papaya seed isolates are the O-H, C=O, C-O, C=C aromatic, C-H aliphatic and C-H aromatic groups.

The results of leaf extract showed absorption at wavenumber 3378.25 cm^{-1} from -OH. The absorption at wavenumbers 2927.58 and 2856.99 cm^{-1} are -CH aliphatic. The absorption at wavenumber 1736.32 cm^{-1} shows C=O. The C=C bond is indicated by absorption at the wavenumber 1623.28 cm^{-1}. The absorption at the waveform region 1385.85 cm^{-1} shows $-CH_3$. Cluster -CO- is indicated by

absorption at wavenumber 1245.42 cm^{-1}. The absorption at wavenumber 1070.01 cm^{-1} shows C-N. So from the infrared spectra data, it shows that papaya leaf extract has functional groups -OH, -CH aliphatic, C-O, C=C, $-CH_3$, -CO-, C-N. Infrared spectra data of papaya leaf isolates may contain several functional groups such as N-O recombination at wavenumber of 3388.05 cm^{-1}, this uptake is supported by the presence of absorption at the 1641.42 cm^{-1} wavenumbers indicating the presence of a bending and at 1017.39 cm^{-1} wavenumbers indicating the presence of CN groups supported also by uptake at wavenumber 1372.68 cm^{-1} showing $-CH_3$ and at wave numbers 606.38 cm^{-1} showing -CH.

After extracts and isolates from papaya seeds and leaves were tested for phytochemicals and their functional groups, the next step was tested for antimicrobial ability, the results are summarized in Table 2.

Table 2. Results of observation of antimicrobial inhibition zone

Antimicrobial source		Type of microbes	Inhibitory zone (mm)		
			Sample	Control (-)	Control (+)
Papaya seeds	Extract	*B. subtilis*	15.9	2.3	10.5
		E. coli	14.3	0.8	9.0
		C. albicans	Resistant	2.4	14.3
	Isolate (Flavonoids)	*B. subtilis*	14.2	2.3	10.5
		E. coli	13.8	0.8	9.0
		C. albicans	Resistant	2.4	14.3
Papaya leaf	Extract	*S. aureus*	14.3	2.1	12.1
		E. coli	11.9	0.8	9.0
	Isolate (Alkaloid)	*S. aureus*	12.6	2.1	12.1
		E. coli	9.7	0.8	9.0

The results of the antibacterial test against *B. subtilis*, *E. coli*, and antifungal against *C. albicans* showed that papaya seed extract was more effective to inhibit the growth of *E. coli* and *B. subtilis* bacteria than the flavonoid isolate. Papaya leaf extract is also more effective in inhibiting the growth of *E.coli* and *S.aureus* bacteria than its alkaloid isolates. The results showed that papaya extract and isolate were more effective in inhibiting the growth of gram-positive bacteria (*B. subtilis* and *S. aureus*) compared to gram-negative bacteria (*E. coli*). The existence of different types of samples in the form of extracts and isolates and types of bacteria suspected to affect antimicrobial activity. In different bacteria produce different inhibitory power. According to Edziri, *et al*., [17] gram-positive bacteria have a single-layer cell wall structure and are composed of peptidoglycan and low-grade lipids (1-4%) so that they are easily penetrated by the flavonoid or alkaloid compounds present in the extract/isolate. While the gram-negative bacteria, has a cell wall structure is layered three and the composition of the cell wall has a high lipid content (11-22%) so difficult to be penetrated by flavonoids or alkaloids contained in polar extract/isolate. This is in accordance with the results of research Wardhani and Supartono [19], which has reported rambutan fruit skin extract more effectively inhibit the growth of *B. subtilis* compared to *E. coli*.

Papaya extract (from seeds and leaves) has a greater inhibitory effect than flavonoids and alkaloids. This is due to the synergistic between the components of the compounds in the extract so as to provide greater antimicrobial activity when compared with isolated flavonoid and alkaloids. Papaya seed extract and isolated flavonoid were inactive to inhibit the growth of *C. albican*s fungus. This is because *C. albicans* has a more complex composition of cell membranes so that the chemicals in extracts and isolates are unable to penetrate the sterol membrane of the cell wall and inhibit chitin synthesis in rigid fungal walls.

4. Conclusion

The active compounds contained in seed extract and papaya leaf are flavonoids, alkaloids, tannins, steroids, and saponins. Extracts and isolated from papaya plants had gram-positive antibacterial activity greater than the gram-negative bacteria, but both did not have antifungal activity. Papaya extracts have

greater antibacterial activity than isolated flavonoid and isolated alkaloid. Strong antibacterial inhibitory sequences are extracts of papaya plants, isolated flavonoid, and isolated alkaloid.

References

[1] A. A. S. K. Darmawati, I. G. A. G. Bawa, and I. W. Suirta, "Isolasi dan Identifikasi Senyawa Golongan Flavonoid pada Daun Nangka (Artocarpus heterophyllus Lmk) dan Aktivitas Antibakteri terhadap Bakteri Staphylococcus aureus," *J. Kim.*, vol. 9, no. 2, pp. 203–210, 2015.
[2] P. Malathi and S. R. Vasugi, "Evaluation of mosquito larvicidal effect of Carica Papaya against Aedes Aegypti," vol. 2, no. 3, pp. 21–24, 2015.
[3] J. A. O. Okeniyi, T. A. Ogunlesi, O. A. Oyelami, and L. A. Adeyemi, "Effectiveness of Dried *Carica papaya* Seeds Against Human Intestinal Parasitosis: A Pilot Study," *J. Med. Food*, vol. 10, no. 1, pp. 194–196, 2007.
[4] N. Artanti, Y. Ma'arifa, and M. Hanafi, "Isolation and Identification of Active Antioxidant Compound from Star Fruit (Averrhoa carambola) Mistletoe (Dendrophthoe pentandra (L.) Miq.) Ethanol Extract," *J. Appl. Sci.*, vol. 6, no. 8, pp. 1659–1663, 2006.
[5] R. S. Sudibyo, "Metabolit Sekunder: Manfaat dan Perkembangannya dalam Dunia Farmasi. Pidato Pengukuhan Jabatan Guru Besar pada Fakultas Farmasi." Fakultas Farmasi Universitas Gadjah Mada, Yogyakarta, 2002.
[6] B. V. Owoyele, O. M. Adebukola, A. A. Funmilayo, and A. O. Soladoye, "Anti-Inflammatory Activities of Ethanolic Extract of Carica Papaya Leaves," *Inflammopharmacology*, vol. 16, no. 4, pp. 168–173, 2008.
[7] J. F. Rehena, "Uji Aktivitas Ekstrak Daun Pepaya (Carica papaya . LINN) sebagai Antimalaria in vitro," *J. Ilmu Dasar*, vol. 11, no. 1, pp. 96–100, 2010.
[8] D. B. S. Utama, Y. M. D. Arina, and M. N. Amin, "Pengaruh Ekstrak Daun Pepaya terhadap Jumlah Sel Limfosit pada Gingiva Tikus Wistar Jantan yang Mengalami Periodontitis," *e-Jurnal Pustaka Kesehat.*, vol. 2, no. 1, pp. 50–57, 2014.
[9] D. V Delphin, R. Haripriya, S. Subi, D. Jothi, and P. T. Vasan, "Phytochemical Screening of Various Ethanolic Seed Extracts," *World J. Pharm. Pharm. Sci.*, vol. 3, no. 7, pp. 1041–1048, 2014.
[10] M. Radji, *Mikrobiologi Panduan Mahasiswa Farmasi & Kedokteran*. Jakarta: Penerbit Buku Kedokteran EGC, 2011.
[11] D. Gould and C. Brooker, *Mikrobiologi Terapan untuk Perawat*. Jakarta: Penerbit Buku Kedokteran EGC, 2003.
[12] Jawetz, J. L. Melnick, and E. A. Adelberg, *Mikrobiologi Kedokteran*. Jakarta: Salemba Medika, 2005.
[13] R. Aksara, W. J. A. Musa, and L. Alio, "Identifikasi Senyawa Alkaloid dari Ekstrak Metanol Kulit Batang Mangga (Mangifera indica L)," *J. Entropi*, vol. 8, no. 1, pp. 514–519, 2013.
[14] J. Alka, K. Padma, and J. Chitra, "Antifungal Activity of Flavonoids of Sida acuta Bum f. Against Candida albicans," *Int. J. Drug Dev. Res.*, vol. 4, no. 3, pp. 92–96, 2012.
[15] M. M. Cowan, "Plant Products as Antimicrobial Agents," *Clin. Microbiol. Rev.*, vol. 12, no. 4, pp. 564–582, 1999.
[16] J. H. Doughari and S. Manzara, "In Vitro Antibacterial Activity of Crude Leaf Extracts of Mangifera indica Linn," *African J. Microbiol. Res.*, vol. 2, no. 2, pp. 67–72, 2008.
[17] H. Edziri, M. Mastouri, M. A. Mahjoub, Z. Mighri, A. Mahjoub, and L. Verschaeve, "Antibacterial, Antifungal and Cytotoxic Activities of Two Flavonoids from Retama raetam Flowers," *Molecules*, vol. 17, no. 6, pp. 7284–7293, 2012.
[18] M. Masibo and Q. He, "In vitro Antimicrobial Activity and the Major Polyphenol in Leaf Extract of Mangifera indica L.," *Malays. J. Microbiol.*, vol. 5, no. 2, pp. 73–80, 2009.
[19] R. A. P. Wardhani and Supartono, "Uji Aktivitas Antibakteri Ekstrak Kulit Buah Rambutan (Nephelium lappaceum L.) pada Bakteri," *Indones. J. Chem. Sci.*, vol. 4, no. 1, pp. 46–51, 2015.