



Proceeding

International Seminar

*Natural Product
for Cancer Chemoprevention*

**Faculty of Pharmacy
Universitas Muhammadiyah Purwokerto
Purwokerto Indonesia, July 5, 2011**

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY PROFILE OF TEMPUYUNG *Sonchus arvensis* L. EXTRACT AND ITS TOXICITY TO *Artemia salina*

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ABSTRACT

Tempuyung (*Sonchus arvensis*) is one of herbal plant which has anticancer potency. The aim of this research were the investigation on anticancer potency of tempuyung based on its toxicity against *Artemia salina* and to obtain high performance liquid chromatography (HPLC) profile of the most toxic tempuyung based on its toxicity on *A. salina*. Tempuyung was collected from 5 different places in Java Island. Each collected sample was extracted with methanol 70% and ethanol 70% by maceration method for 3 hours. Phytochemical test results showed that tempuyung contained saponins, flavonoids, steroids, and tannins. Toxicity results against *A. salina* showed that LC₅₀ of tempuyung 70% ethanol extract (325.63 ppm) was more toxic than 70% methanol extract (766.44 ppm). HPLC profile of tempuyung from Solo ethanol 70% extract was performed by isocratic and gradient method using methanol-acetic acid 0.01% (v/v) as the mobile phase. Based on the chromatogram, in the gradient method 3–7 peaks were obtained, therefore it showed better profile for fingerprint compared with isocratic method which produced only 1–4 peaks.

Keywords: *Sonchus arvensis*, HPLC, toxicity, *Artemia salina*

Introduction

Tempuyung (*Sonchus arvensis*) is one of Indonesian medicinal plant which has potency to treat gout, diuretic, kidney stones, coughing, asthma, bronchitis, and has potency as anticancer. The potency of tempuyung as anticancer came from its secondary metabolites such as alkaloids, flavonoids, saponins, and tannins which often used in medicines (Khurniasari 2004). To find its activity as anticancer, firstly the samples tested with Brine Shrimp Lethality Test (BSLT) which is used *Artemia salina* larvae as animal test. The BSLT

results data has been shown have correlation with cyto-toxicity and anticancer compounds (Meyer *et al* 1982).

The consistency of medicinal plants material can be determined by HPLC methods. This method can be used for analysis of purity, active components and also for profile analysis. HPLC profile analysis used for quality control of herbal medicines because it can explain the characteristics of herbal medicine in comprehensive manner. The informative and preventative profiles can be obtained through the optimization of mobile phase in HPLC

system. The aim of this research were to investigate the anticancer potency of tempuyung based on its toxicity against *Artemia salina* and to obtain high performance liquid chromatography (HPLC) profile of the most toxic tempuyung based on its toxicity on *A. salina*.

Material and methods

This research consisted of three steps. The first step consisted of collecting samples from different area in Java Island, preparation of samples, extraction, and phytochemical content test. The second step consisted of BSLT test with statistical analysis, and the third step consisted of HPLC optimization analysis.

Tempuyung samples were collected from Pusat Studi Biofarmaka (Bogor 1), Gunung Batu (Bogor 2), Balitro. (Bogor 3), Solo, and Wonogiri. Each sample together with *Vinca rosea* leaves collecting from Bogor 1 were dried and powdered to make it as simplicia (Djamal 1990). All of simplicia were extracted with methanol 70% and ethanol 70% (Suwandi 2008) with maceration method. The phytochemical content analysis was performed with Harbone (1987) methods. BSLT analysis were performed with Juniarti et al (2009) method, continue with statistical analysis with Duncan test.

HPLC analysis was performed to tempuyung extract which has the lowest LC50 data from BLST test. The HPLC condition was using HPLC Shimadzu LC-20A, column C18 (150 mm x 4.6 mm i.d, particle size 5 μ m. Gradient elution with methanol – acetic acid 0.01% (v/v) was applied with flow rate 1mL/min. Detection with UV-Vis detector at 350, 360, and 662 nm.

Results and discussion

Samples from 5 different regions had different water content and extraction yield (Table 1). The lowest yield was found at tempuyung from Wonogiri which only has 3.44% for methanol 70% extract and 3.22% for ethanol 70% extract yield. Although the yield of each sample different, but based on the simplicia phytochemical content, each simplicia of tempuyung has the same phytochemical content. The phytochemical content of tempuyung is different from Tapak Dara. The differences were on triterpenoid and steroid content. Tapak dara did not show the positive result on triterpenoid and steroid while tempuyung showed it (Table 2).

Table 1 Extraction yield of tempuyung and tapak dara

Sample	Water content (%)	Extract yield (%b/b) ± SD	
		MeOH 70%	EtOH 70%
Tempuyung			
Bogor 1	9.53 ± 0.10 _b	5.78 ± 0.20 _{cd}	7.80 ± 0.24 _e
Bogor 2	10.64 ± 0.10 _d	6.48 ± 0.20 _d	6.27 ± 0.20 _d
Bogor 3I	9.27 ± 0.23 _b	6.11 ± 0.10 _{bc}	5.56 ± 0.10 _c
Solo	8.25 ± 0.08 _a	5.48 ± 0.07 _b	4.36 ± 0.06 _b
Wonogiri	9.59 ± 0.10 _b	3.44 ± 0.38 _a	3.22 ± 0.24 _a
Tapak dara			
Bogor I	9.59 ± 0.10 _{bc}	10.25 ± 0.07 _e	14.32 ± 0.11 _f

Note: Different alphabet mean significant different at p < 95%. (Duncan test)

Table 2 Phytochemical content analysis of tempuyung from Solo and tapak dara from Bogor 1

Phytochemical test	Result	
	Tempuyung	Tapak Dara
Alkaloid	-	++
Saponin	+	+
Flavonoid	++	+
Triterpenoid	+	-
Steroid	+	-
Tannin	+	+

Note: (+) shows the color intensity, (-) shows no color (no results)

The toxicity of all extracts against *Artemia salina* were tested with BSLT test. The results showed in Table 3. Tempuyung extracts were not as toxic as tapak dara. Tapak dara extract gave LC50 lower than all extracts of tempuyung, LC50 729 ppm for methanol extract and 310 ppm for ethanol extract. Between all tempuyung extract, tempuyung extract from Solo gave the lowest LC50 value. It means that the most toxic tempuyung extract was tempuyung extract from Solo. It gave LC50 value 766 ppm for methanol extract and 326 ppm for

ethanol extract. Although the LC50 value of tempuyung extract from Solo significantly different with LC50 value of tapak dara, but according to Meyer et al (1982) and Anderson (1991), tempuyung extract still have potency as anticancer.

The LC50 value of tempuyung ethanol 70% extract is better than kamanggi extract (LC50 16182 ppm, Muktar et al 2007) and saga leaves (LC50 607ppm, Juniarti et al 2007). Based on this results, HPLC profile for tempuyung performed with tempuyung extract from Solo.

Table 3 LC₅₀ data of metanol 70% and etanol 70% extract of tempuyung and tapak dara

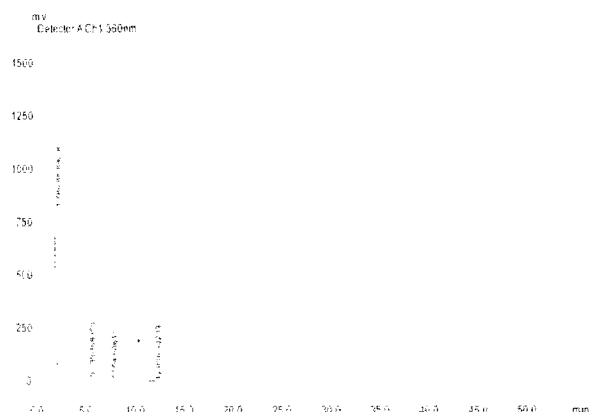
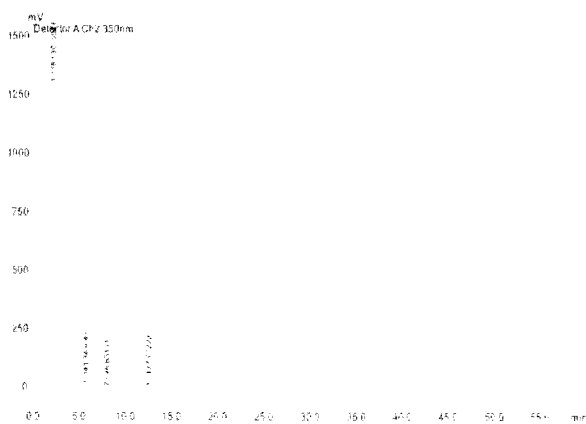
Extract	LC ₅₀ (ppm)	
	Methanol 70%	Ethanol 70%
Tempuyung		
Bogor 1	1934.47 ± 8.08 _f	410.07 ± 3.89 _d
Bogor 2	1010.51 ± 9.77 _c	449.76 ± 5.50 _f
Bogor 3	1107.34 ± 12.74 _e	426.52 ± 3.31 _e
Solo	766.44 ± 20.63 _b	325.63 ± 4.94 _b
Wonogiri	1020.89 ± 0.14 _{cd}	386.61 ± 9.97 _c
Tapak dara		
Bogor 1	729.48 ± 4.36 _a	310.34 ± 3.53 _a

Note: Different alphabet mean significant different at p < 95%. (Duncan test)

The HPLC profile analysis initially performed using acetonitrile and methanol as mobile phase in isocratics system. Both solvents resulted only small number of peaks. It means

the conditions did not produce a good profile. This may be due to less polar mobile phase making it less able to carry the component. Furthermore, the analysis was done with mixture solvent (methanol and acetic acid 0.01% (v/v)). The number of peaks that can be detected was calculated based on the criteria of the resolution and signal to noise ratio (S/N). The recognized peak was counted on resolution value ≥ 3.

The analysis of the chromatogram was detected with UV-Vis detector at 350, 360 and 662 nm. The chromatograms for isocratic elution produced also only small number of peaks (Fig 1). It means that this condition is not good for profile. To find better condition, gradient elution was performed. The increasing of solvent polarity can produce the shorter retention time of compounds which strongly retained in column.



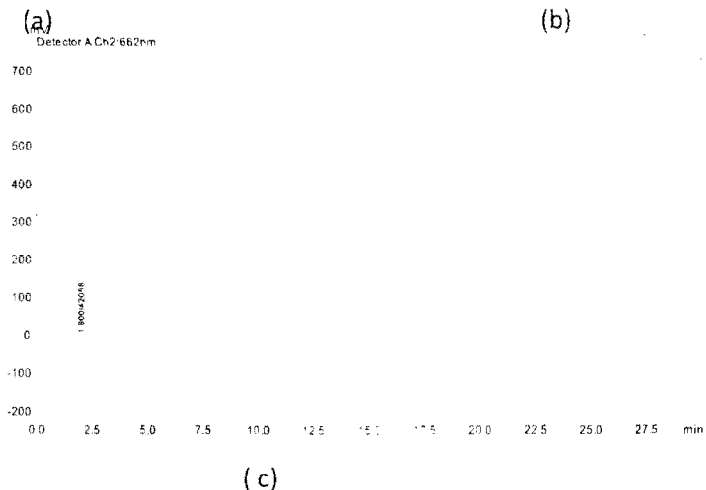
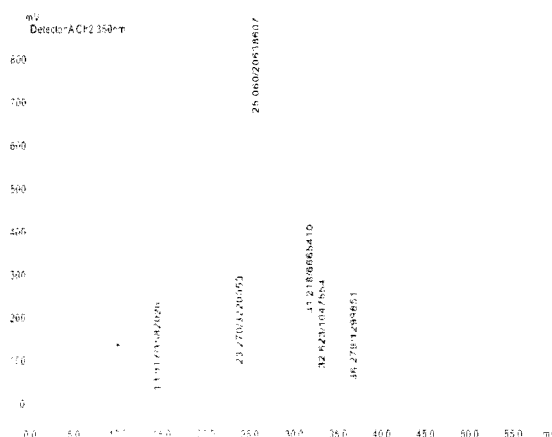
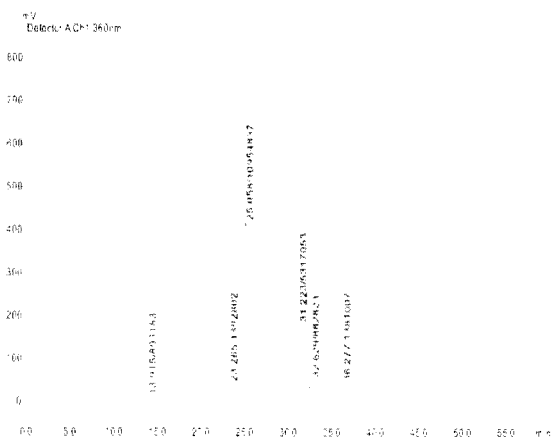


Fig 1. Chromatogram of tempuyung ethanol extract with isocratic condition (methanol:acetic acid 0.01%) at 350 (a), 360 nm (b), and 662 nm (c).

The chromatogram of tempuyung ethanol extract at gradient elution is shown in Fig 2. This condition generated more number of peaks compared to isocratic elution method. It produced about 7 peaks which do not appear only in the early minutes, but emerged during the analysis lasted until the 55 minutes. Thus, the profile formed by the gradient method is

more representative than the isocratic method. Tempuyung contained of flavonoids such as flavones, flavonols, and khalkon (Sriningsih et al 2005). According to Markham (1988), this group of components absorbs UV at 350 – 360 nm. Therefore, profile determination of tempuyung ethanol extract will better perform at 350 – 360 nm.



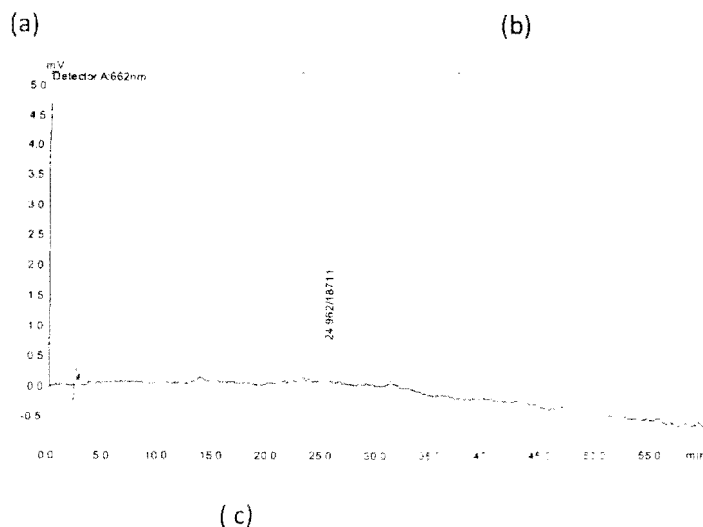


Fig 2. Chromatogram of tempuyung ethanol extract with gradient condition (methanol:acetic acid 0.01%) at 350 (a), 360 nm (b), and 662 nm (c).

Conclusion

Tempuyung ethanol extract had potency as anticancer. From 5 different location of tempuyung, the most prospective tempuyung was tempuyung from Sojo with LC50 value 325 ppm. Flavonoid contained in tempuyung extract included flavones, flavonols, and khalkon. The best HPLC chromatogram profile obtained using gradient elution method with UV detection at 350 nm.

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