Title: Screening of extraction method for alkaloid enrichment of *Peperomia pellucida* (L.) Kunth
Name & mail address of corresponding author: Islamudin Ahmad (islamudinahmad@yahoo.com)
Abdul Mun'im (munimabdoel@gmail.com)

Highlighted Corrections

S.No.	Asked query no.	Details
1	AQ1	Rissyelly is a real full name
2	AQ2	Tarigan IM, Bahri S, Saragih A. Antihyperuricemia activity of Suruhan (Peperomia pellucida (L.) Kunth) herb ethanol extract on Mus musculus male. J. Pharm Pharmacol. 2012; 1(1): 37-43 (In bahasa)

Other corrections

Page No.	Column(left or right)/Section /Paragraph/line no or table or figure	Incorrect text or matter	Corrected text
1	Paragraph Conclucion/ line 26-27	these herbs.	this herb.
5	Column (left)/line (68-69)	pipeline	piperine
5	Column (right)/line (22-24)	Tarigan IM, Bahri S, Saragih A. Aktivitas	Tarigan IM, Bahri S, Saragih A. Antihyperuricemia

		antihiperurisemia ekstrak etanol herba suruhan (Peperomia pellucida (L). Kunth) pada mencit Jantan. J Pharm Pharmacol 2012;1(1):37-43	activity of Suruhan (Peperomia pellucida (L.) Kunth) herb ethanol extract on Mus musculus male. J. Pharm Pharmacol. 2012; 1(1): 37-43 (In bahasa)
5	Column (right)/line (33-34)	De Fátima Arrigoni-Blank M	Arrigoni-Blank FM

SCREENING OF EXTRACTION METHOD FOR ALKALOID ENRICHMENT OF *PEPEROMIA PELLUCIDA* (L.) KUNTH

ISLAMUDIN AHMAD[1,2]*, RISSYELLY, AGUS KURNIAWAN[3], ABDUL MUN'IM[2]*

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Mulawarman University, Samarinda, East Kalimantan, Indonesia. [2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Indonesia, Depok 16424 West Java, Indonesia. [3]Department of Pharmacy, Faculty of Medicine and Health, Gunadharma University, Depok, West Java, Indonesia.
Email: islamudinahmad@yahoo.com/munimabdoel@gmail.com

ABSTRACT

Objective: The aims of this study were to investigate the effect of extraction method modification of *Peperomia pellucida* (L.) Kunth herbs for alkaloid enrichment and thin layer chromatography (TLC) profile.

Methods: The dried herbs were extracted with six different methods (including; method A, B, C, D, and E). The obtained extract solution was evaporated and was analyzed. Alkaloid content identified using Dragendorff spray reagent. The gravimetric method was used to determine of total alkaloid, and TLC profile was analyzed using a densitometer at a wavelength of 254 nm and 366 nm.

Results: The results showed a difference from all modification of extraction method for alkaloid enrichment and their TLC profile. The use of eluent dichloromethane (DCM):methanol (95:5) was potential for isolation of the compound. From whole extraction methods, only one that tested positive for alkaloids was method A. Total alkaloids in DCM fraction amounted to 29.59 mg/g piperine.

Conclusion: Based on the above results, method A can be applied for isolation of alkaloid compound from the herbs.

Keywords: *Peperomia pellucida* (L.) Kunth, Extraction method, Alkaloid enrichment, Thin layer chromatography profile.

INTRODUCTION

Peperomia pellucida (L.) Kunth is herbaceous of Piperaceae family. It can be found in places such as under the wall, on the ground side of the road, and under the tree. This herb traditionally used to treat a headache accompanied by fever, abdominal pain, hypertension, and diabetes mellitus [1,2]. The use as diabetes mellitus has demonstrated in the previous studies [3]. The ethanol extract of *P. pellucida* herbs has proven to have gout activity in mice and rats [4]. Moreover, this herb also has anti-inflammatory, analgesic, antipyretic activity [3,5], and angiotensin converting enzyme inhibitors [6,7].

P. pellucida herb contains flavonoids, phenols, terpenes, and essential oil [8,9]. Some of the new compounds have been isolated, namely, two secolignan, two tetrahydrofurans, dihydronaftalenon methoxilation [10], pelucidin A, dilapiole [11], and chromene [12]. Many studies reported that the herb contains alkaloids [13-18]. However, no data from studies that reported the results of the isolation and characterization of alkaloid compounds found in *P. pellucida* herbs. Therefore, we were interested in extraction and isolation of alkaloids from this herb. Isolation of alkaloids is not easy because it desperately needed a special method for separating alkaloids from plants in contrast to the isolation of the compounds exist in general. Based on the above reason, we carried out a study to find the optimal extraction method which can be applied for alkaloid enrichment in the resulting extract.

MATERIALS AND METHODS

Plant materials

P. pellucida herbs were collected from Bogor Botanical Garden, Indonesian Institute of Sciences. The voucher specimens identified at the Herbarium Bogoriense, Bogor, West Java, Indonesia and were deposited at Pharmacognosy-Phytochemistry Laboratory, Universitas Indonesia, West Java, Indonesia.

Chemical material and general equipment

The used chemical in this study included N-hexane, dichloromethane (DCM), methanol (MeOH), chloroform (CHCl$_3$). Toluene (analytical grade), acetone (analytical grade), ethanol (analytical grade), ethyl acetate (analytical grade) were purchased from Smart Lab Indonesia. CHCl$_3$ (analytical grade), 25% NH$_4$OH (analytical grade), and HCl (analytical grade), sodium carbonate, disodium sulfide, thiourea were purchased from Merck, Germany, TLC Silica Gel 60 F$_{254}$ (Merck, Germany). Moreover, the used equipment included chamber and densitometer Camaq Wincat 3 TLC Scanner (Camaq, Switzerland); a rotary evaporator (Buchi, Germany); spectrophotometer UV-VIS (Thermo Scientific, USA); vortex mixer (WiseMix® VM-100, Korea), as well as equipment for maceration and soxhlet extraction.

Extraction methods

Method A

Extraction with maceration using acid-base extraction method reported by Tiong *et al.* [19], with modification. Dried *P. pellucida* herbs were macerated with n-hexane for 3 days at room temperature, and the process was repeated twice. After removal of solvent, the residue was firstly soaked with 25% ammonia for an hour, followed by soaking with DCM. The DCM extract (DE) obtained after filtered and dried under reduced pressure. DE was subjected to acid-base extraction using 5% hydrochloric acid and 25% ammonia solution to obtain of crude alkaloid.

Method B

Dried *P. pellucida* herbs were extracted with Soxhlet using MeOH (CH$_3$OH) several times; MeOH extract (ME) solution was evaporated using rotary vacuum evaporator. ME produced and applied for the next fractionation process. The ME was dissolved in 0.5 M HCl then was put into a separatory funnel and washed with CH$_2$Cl$_2$. The CH$_2$Cl$_2$ layer

was separated (fraction 1) and then acid layer washed with CH_2Cl_2 and basified to pH 9 using 25% ammonia solution. Obtained CH_2Cl_2 layer was separated (fraction 2) and then alkali layer was washed with CH_2Cl_2 and basified to pH 11 (25% ammonia) to give CH_2Cl_2 layer (fraction 3) and alkali layer. Fraction 1, 2, 3 contain alkaloid (if any).

Method C

Extraction with maceration using acid-base extraction method by Lebrini *et al.*, 2011 [20] with modification. Briefly, dried *P. pellucida* herbs were treated with alkaline solutions (5%NH_4OH). The alkalized raw material was extracted with DCM (3×200 mL). Filtration collected the extract and the combined DCM fractions were washed with water (2×200 mL). Then, the organic layer was extracted three times with diluted HCl. The combined aqueous fractions were washed with DCM (3×80 mL), and the pH of the aqueous solution adjusted to 9 with 25% NH_4OH. This aqueous layer was extracted with DCM (3×100 mL), and the combined organic layers were washed with distilled water (2×100 mL), dried over Na_2SO_4, and collected by filtration. The solvent was removed under reduced pressure to give the alkaloid (if any).

Method D

Extraction with reflux using dilute acid solution. The dried *P. pellucida* herbs were refluxed using 0.5 N HCl and heated for a few minutes. Moreover, then was filtered and separated from solution and residue. The acid solution was extracted with DCM for three times. Formed two layers, namely, DCM layer (1) and acid layer (2). DCM layer (1) basified using 1-2% NH_4OH, then formed two layers is a layer of DCM (fraction 1) and the base layer. The base layer was basified again with 25% NH_4OH and extracted with DCM and DCM layer was separated (fraction 2). The next layer is the acid (2) was added ammonia solution to pH 9-10 and extracted with DCM. DCM layer was basified with NaOH and then separated again. DCM layers were dried (fraction 3).

Method E

Acid-base extraction method using Wagner method [21] with slight modification. Briefly, dried *P. pellucida* herbs were shaken for 15 minutes with 0.1 N sulfuric acid and then filtered. The filter was washed with 0.1 N sulfuric acid; concentrated ammonia then added. The mixture shakes with two portions of diethyl ether. The ether was dried over anhydrous $NaSO_4$, filtered and evaporated to dryness and the residue was dissolved in MeOH.

Determination of thin layer chromatography (TLC) profile

TLC profile determination was conducted by TLC method using TLC plates as the stationary phase and eluent comparison based on the level of polarity as the mobile phase. TLC profile analysis using a densitometer (Camag Wincat TLC Scanner 3) at a wavelength of 254 nm and 366 nm [22,23].

Alkaloid identification

Identification of alkaloid was performed using the Dragendorff spray reagent. Reagent sprayed on the TLC plate which eluted with an appropriate eluent. Reaction tested positive if the color changes to orange-brown on particular spots [24,25].

Calculation of total alkaloids

The method was used to calculating the total alkaloid extract refer to a Sreevidya and Mehrotra, 2003 [26] method where using the gravimetric method. The principle of the gravimetric method is the formation of precipitate complexes by reagents that react with alkaloid like Dragendorff reagent. Theses precipitate measured with visible light 435 nm using spectrophotometry.

RESULTS AND DISCUSSION

Alkaloids are secondary metabolites whose presence in a plant species is very limited and has limited distinguish feature of each

plant. Alkaloids extraction is not easy when it needs special methods to attract alkaloids contained in plants. Based on several studies has reported that contains alkaloid [13-18], but, until now, there have been no reported studies related to the isolation and characterization of alkaloid compounds contained in the plant. In this research, the effect of an extraction method for enrichment and their TLC profile. However, not only the class of alkaloids but also all the compounds contained in extracts of this plant are based on profiles TLC. The objective of our study was to determine the effect of extraction methods for the alkaloid enrichment and its TLC profile.

In this study, the extraction was conducted using five kinds of modification of the extraction method using 100 g of dried samples (each method). From the extraction method was obtained for each extract namely, method A resulted in 24.63 g, method B was obtained three fractions namely fraction extract B1 (20.14 g), B2 (18.86 g) and B3 (10.05 g), method C was 23.17 g, method D was obtained two fractions of each extract D1 (12.34 g), D2 (7.05 g), and method E (extract E) was 5.32 g. The use and modification of the method are carried out with the aim to minimize chlorophyll compounds contained in these desired plants extract.

Determination profile TLC

TLC profile determination was conducted using the TLC plate that has activated. The eluent used three kinds, such as the combination of DCM: MeOH (95:5), toluene: ethyl acetate (70:30), and toluene: acetone:ethanol:25% ammonia (20:20:3:1). Each combination of the eluent was analyzed using a densitometer at a wavelength of 254 nm and 366 nm (Fig. 1).

As can be seen in Table 1 showed, the deployment of the highest number of spots, namely, the method A and C was 22 spots; the method D (two fractions) was 22 (D1) and 18 (D2) spots; the method B (three fractions) was total spots of 19 (B1), 15 (B2), and 17 (B3); the method E was 15 spots, whereas for the control of the Piperine (three spots) and spot spread use than any other comparison eluent.

In Table 2 shows, the deployment of the highest number of spots was 16 spots (method C), 15 spots (method B, fraction 1 and 2), 14 spots (method A), 13 spots (method E), 12 spots (method D, fraction 1), 8 spots (method B, fraction 3), and 7 spots (method D, fraction 2), respectively, whereas for the control of piperine (three spots).

In Table 3 shows, the spread of the highest number of spots was method D (fraction 1; 14 spots), method B and A (10 spots), and methods C and E (8 spots), while the control of Piperine (three spots). It shows that all the modifications methods obtained TLC profile different based on the difference polarity of the compound were attracted to each kind of eluent combinations. Moreover, also based on the Rf value for each combination of eluent used to show that the combined use of eluent DCM: MeOH (95:5) (Table 1) have the potential for further study especially isolation of the target compound because the separation spots were obtained the best spread.

Alkaloids identification

P. pellucida contains alkaloids using reagents Dragendorff to see the results formed of brownish orange precipitation. While the identification of alkaloids in this study was performed using reagents Dragendorff spray that sprayed on the TLC spot outcome from each extract. However, the five methods were only one who tested positive for alkaloids namely method A than the control positive of piperine (Fig. 2). The results do not ensure their continued with improvements in the identification and enrichment of alkaloids from this plant but it may alsWo be influenced by place to grow plants that affected the content of alkaloids or the extraction method used has not maximized.
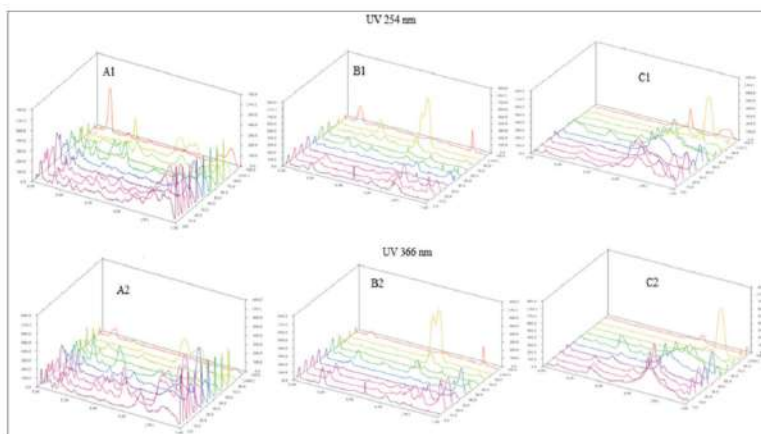
Fig. 1: Thin layer chromatography (TLC) profile using densitometry TLC scanner, (A) using dichloromethane:methanol eluent (95:5), (B) using toluene:ethyl acetate (7:3), (C) using toluene:acetone:ethanol:25% ammonia (20:20:3:1)

Table 1: TLC profiles using dichloromethane:methanol eluent (95:5)

Extract	Wavelength (nm)	No. of peaks	Rf values	Percentage peaks area
A	254	22	0.04, 0.07, 0.10, 0.14, 0.16, 0.24, 0.29, 0.34, 0.36, 0.39, 0.44, 0.51, 0.53, 0.56, 0.57, 0.62, 0.64, 0.70, 0.81, 0.89, 0.90, 0.93	8.80, 0.47, 0.79, 9.24, 2.77, 6.39, 3.19, 2.69, 2.25, 2.44, 7.88, 0.022, 0.57, 1.14, 0.76, 1.76, 0.81, 16.22, 24.82, 3.37, 2.77, 0.66
	366	20	0.04, 0.06, 0.10, 0.14, 0.16, 0.19, 0.24, 0.34, 0.39, 0.45, 0.46, 0.52, 0.57, 0.62, 0.70, 0.78, 0.81, 0.86, 0.87, 0.89	7.26, 5.17, 2.13, 11.54, 2.59, 1.61, 2.96, 1.46, 0.86, 2.27, 1.83, 0.45, 0.59, 1.10, 34.34, 1.83, 0.82, 11.43, 3.27, 6.32
B1	254	19	0.04, 0.06, 0.09, 0.12, 0.17, 0.21, 0.30, 0.32, 0.38, 0.44, 048, 0.55, 0.60, 0.65, 0.71, 0.80, 0.86, 0.92	4.42, 0.54, 0.56, 6.02, 3.13, 1.93, 5.15, 2.76, 2.76, 10.94, 2.82, 0.69, 8.08, 5.01, 1.28, 2.05, 10.23, 26.96, 7.41
	366	14	0.04, 0.06, 0.09, 0.12, 0.16, 0.21, 0.33, 0.38, 0.44, 0.54, 0.61, 0.79, 0.83, 0.92	2.28, 1.08, 1.07, 7.13, 1.69, 0.76, 5.55, 4.25, 3.88, 21.18, 6.30, 20.88, 12.16, 10.79
B2	254	15	0.04, 0.06, 0.09, 0.12, 0.16, 0.20, 0.28, 0.35, 0.37, 0.41, 0.49, 0.60, 0.69, 0.85, 0.92	4.61, 0.42, 0.32, 8.87, 3.32, 1.55, 2.59, 6.46, 2.58, 3.99, 6.77, 3.60, 0.60, 42.03, 11.95
	366	16	0.04, 0.06, 0.09, 0.12, 0.16, 0.21, 0.24, 0.30, 0.36, 0.38, 0.41, 0.49, 0.60, 0.76, 0.81, 092	2.28, 0.69, 0.55, 7.05, 2.01, 0.75, 0.23, 2.85, 2.54, 3.04, 6.38, 15.14, 5.51, 15.75, 19.69, 15.53
B3	254	17	0.04, 0.08, 0.13, 0.16, 0.24, 0.28, 0.32, 0.39, 0.39, 0.41, 0.47, 0.52, 0.64, 0.67, 0.74, 0.79, 0.92	14.00, 3.51, 24.93, 5.52, 1.59, 4.43, 2.82, 3.34, 1.86, 2.45, 7.72, 0.74, 1.08, 2.43, 1.46, 11.12, 11.00
	366	14	0.04, 0.06, 0.08, 0.12, 0.16, 0.30, 0.33, 0.39, 0.47, 0.57, 0.59, 0.74, 0.79, 0.91	4.81, 1.10, 0.91, 19.10, 2.21, 5.31, 0.82, 5.15, 8.93, 1.80, 0.84, 6.99, 14.06, 27.96
C	254	23	0.04, 0.09, 0.12, 0.15, 0.20, 0.24, 0.30, 0.33, 0.37, 0.41, 0.43, 0.47, 0.48, 0.51, 0.53, 0.56, 0.59, 0.60, 0.62, 0.68, 0.75, 0.85, 0.92	6.15, 0.39, 11.18, 7.19, 1.83, 0.75, 6.95, 2.96, 5.74, 0.29, 0.70, 2.18, 1.36, 1.18, 0.96, 0.95, 3.47, 1.27, 0.83, 1.87, 1.38, 19.02, 21.41
	366	13	0.04, 0.10, 0.12, 0.18, 0.30, 0.33, 0.38, 0.47, 0.53, 0.59, 0.75, 0.80, 0.92	3.98, 0.67, 6.98, 2.91, 5.19, 0.91, 4.01, 9.49, 0.40, 3.29, 7.05, 17.68, 37.54
D1	254	18	0.03, 0.04, 0.08, 0.10, 0.13, 0.20, 0.26, 0.32, 0.35, 0.40, 0.52, 0.61, 0.67, 0.69, 0.74, 0.79, 0.85, 0.94	0.54, 2.04, 0.73, 0.39, 12.02, 7.16, 1.56, 22.06, 12.94, 20.87, 0.33, 5.76, 1.67, 1.33, 0.65, 5.18, 3.16, 1.68
	366	10	0.04, 0.08, 0.14, 0.20, 0.32, 0.40, 0.61, 0.79, 0.84, 0.92	0.70, 4.52, 16.36, 1.51, 27.22, 1.80, 14.66, 25.88, 3.22, 4.14
D2	254	20	0.04, 0.14, 0.17, 0.23, 0.26, 0.29, 0.30, 0.34, 0.36, 0.45, 0.46, 0.49, 0.51, 0.64, 0.75, 0.77, 0.80, 0.80, 0.84, 0.88	20.25, 24.00, 2.69, 0.87, 2.75, 2.97, 5.12, 1.32, 13.73, 1.70, 0.92, 1.66, 1.89, 0.68, 1.18, 2.84, 2.22, 1.48, 0.99, 10.74
	366	10	0.04, 0.14, 0.24, 0.29, 0.36, 0.45, 0.55, 0.77, 0.81, 0.88	25.85, 22.15, 2.15, 4.31, 7.32, 0.96, 1.71, 3.84, 22.71, 9.00
E	254	15	0.05, 0.16, 0.18, 0.27, 0.32, 0.36, 0.39, 0.46, 0.50, 0.62, 0.66, 0.70, 0.82, 0.90	5.60, 6.65, 5.38, 7.80, 11.83, 0.97, 18.75, 1.32, 7.13, 1.12, 0.95, 23.10, 5.15, 4.25
	366	12	0.05, 0.15, 0.19, 0.22, 0.32, 0.36, 0.39, 0.50, 0.64, 0.70, 0.83, 0.87	5.06, 12.84, 6.03, 1.12, 3.04, 1.26, 9.37, 0.93, 1.45, 5.15, 41.54, 12,21
p	254	4	0.33, 0.65, 0.71, 0.76	18.66, 42.89, 13.08, 25.37
	366	2	0.65, 0.76	52.71, 47.29

A - A method. B - B methods (3 fraction). C - C method. D - D methods (2 fraction). E - E method. piperine (control). (254 and 366) wavelength at 254 and 366 nm. TLC: Thin layer chromatography

Table 2: TLC profiles using toluene:ethyl acetate eluent (7:3)

Extract	Wavelength (nm)	No. of peaks	Rf values	Percentage peaks area
A	254	14	0.05, 0.12, 0.18, 0.27, 0.30, 0.38, 0.44, 0.47, 0.52, 0.53, 0.60, 0.70, 0.77, 0.92	0.59, 4.48, 16.14, 1.13, 3.66, 3.80, 3.00, 9.54, 4.15, 4.37, 12.92, 2.46, 33.31, 0.46
	366	13	0.12, 0.18, 0.39, 0.40, 0.47, 0.52, 0.58, 0.62, 0.68, 0.75, 0.80, 0.92	2.68, 38.56, 3.54, 5.55, 10.82, 2.65, 1.14, 16.60, 4.07, 9.32, 3.03, 1.05
B1	254	15	0.18, 0.30, 0.36, 0.37, 0.40, 0.51, 0.59, 0.63, 0.68, 0.71, 0.74, 0.80, 0.83, 0.96	14.16, 0.87, 2.24, 2.20, 1.62, 4.55, 2.35, 7.14, 9.85, 2.17, 30.68, 2.03, 5.26, 2.94, 11.85
	366	9	0.18, 0.51, 0.62, 0.68, 0.71, 0.74, 0.80, 0.83, 0.96	11.87, 4.10, 5.43, 10.50, 2.96, 19.47, 8.53, 11.79, 25.35
B2	254	15	0.19, 0.27, 0.29, 0.36, 0.41, 0.52, 0.59, 0.63, 0.68, 0.74, 0.80, 0.83, 0.87, 0.90, 0.96	11.97, 1.23, 1.47, 2.01, 3.42, 6.48, 7.34, 8.96, 11.33, 2.48, 6.62, 0.48, 3.74, 1.96
	366	11	0.18, 0.38, 0.41, 0.51, 0.61, 0.67, 0.74, 0.80, 0.83, 0.87, 0.96	11.24, 1.08, 2.40, 4.02, 10.66, 10.90, 12.94, 9.68, 14.21, 0.30, 22.57
B3	254	8	0.17, 0.18, 0.60, 0.61, 0.66, 0.70, 0.74, 0.78	4.91, 8.16, 21.99, 6.16, 19.5, 14.80, 14.50, 9.96
	366	5	0.18, 0.78, 0.82, 0.92, 0.96	18.60, 12.90, 17.70, 4.47, 46.33
C	254	16	0.06, 0.08, 0.16, 0.19, 0.28, 0.30, 0.36, 0.40, 0.52, 0.59, 0.62, 0.68, 0.73, 0.82, 0.83, 0.96	1.79, 4.17, 3.60, 5.37, 5.33, 4.06, 1.34, 5.44, 1.94, 9.88, 7.99, 16.49, 27.23, 1.80, 2.65, 0.92
	366	7	0.19, 9.61, 0.66, 0.74, 0.79, 0.83, 0.97	10.79, 10.12, 5.08, 7.07, 4.79, 8.83, 53.31
D1	254	12	0.06, 0.15, 0.20, 0.28, 0.37, 0.37, 0.40, 0.54, 0.61, 0.73, 0.82, 0.87, 0.95	1.46, 0.93, 24.29, 11.06, 9.18, 8.72, 1.49, 21.76, 11.91, 0.89, 2.55, 5.77
	366	11	0.06, 0.20, 0.37, 0.39, 0.61, 0.66, 0.73, 0.77, 0.87, 0.96, 0.97	4.77, 16.12, 3.49, 9.02, 46.10, 3.14, 2.34, 4.88, 1.69, 4.49, 3.95
D2	254	7	0.09, 0.15, 0.17, 0.20, 0.28, 0.58, 0.74	4.73, 4.21, 11.13, 17.91, 5.00, 37.70, 19.31
	366	6	0.17, 0.20, 0.58, 0.61 0.74, 0.96	5.26, 18.31, 13.94, 30.08, 20.67, 11.74
E	254	13	0.03, 0.09, 0.13, 0.21, 0.29, 0.37, 0.44, 0.54, 0.59, 0.68, 0.74, 0.85, 0.94	2.37, 1.55, 4.31, 5.88, 13.49, 13.75, 5.98, 5.74, 31.37, 2.65, 6.29, 1.95, 4.67
	366	9	0.03, 0.09, 0.12, 0.20, 0.62, 0.66, 0.74, 0.77, 0.96	1.06, 1.27, 5.62, 5.37, 52.12, 8.97, 5.87, 10.81, 8.90
P	254	3	0.30, 0.59, 0.63	14.79, 29.80, 55.42
	366	3	0.29, 0.59, 0.63	1.16, 44.12, 54.72

A - A method, B - B methods (3 fraction), C - C method, D - D methods (2 fraction), E - E method, and piperine (control), (254 and 366) wavelength at 254 and 366 nm. TLC: Thin layer chromatography

Table 3: TLC profiles using toluene:acetone:ethanol:25% ammonia eluent (20:20:3:1)

Extract	Wavelength (nm)	No. of peaks	Rf values	Percentage peaks area
A	254	10	0.08, 0.26, 0.31, 0.39, 0.52, 0.68, 0.71, 0.74, 0.88, 0.97	1.53, 1.04, 2.73, 1.37, 2.97, 2.39, 15.12, 30.82, 17.36, 1.68
	366	8	0.08, 0.26, 0.31, 0.52, 0.71, 0.82, 0.88, 0.96	1.91, 1.19, 9.09, 2.75, 64.23, 14.29, 6.38, 0.17
B1	254	9	0.06, 0.28, 0.30, 0.53, 0.68, 0.70, 0.78, 0.90, 0.98	1.21, 5.17, 7.98, 1.69, 30.70, 19.66, 15.50, 14.92, 3.18
	366	8	0.13, 0.24, 0.29, 0.52, 0.68, 0.89, 0.94, 0.97	1.26, 1.26, 10.74, 4.13, 7517, 3.45, 1.95, 2.05
B2	254	10	0.03, 0.25, 0.52, 0.57, 0.66, 0.69, 0.76, 0.89, 0.97	0.44, 5.56, 2.44, 2.06, 30.65, 17.13, 8.38, 12.55, 15.77, 5.02
	366	10	0.03, 0.12, 0.25, 0.51, 0.67, 0.69, 0.80, 0.88, 0.93, 0.97	0.40, 0.72, 10.26, 5.56, 45.03, 26.99, 3.88, 2.65, 1.10, 3.39
B3	254	10	0.03, 0.09, 0.12, 0.33, 0.43, 0.57, 0.66, 0.69, 0.74, 0.98	0.91, 0.85, 1.61, 6.59, 2.31, 4.34, 30.94, 24.49, 21.65, 6.32
	366	9	0.03, 0.33, 0.51, 0.57, 0.66, 0.69, 0.81, 0.87, 0.97	1.01, 14.14, 2.63, 2.19, 44.12, 22.64, 3.63, 1.90, 7.72
C	254	8	0.34, 0.42, 0.44, 0.64, 0.69, 0.72, 0.88, 0.97	0.83, 0.66, 0.27, 29.08, 23.87, 29.10, 12.82, 3.39
	366	7	0.4, 0.43, 0.51, 0.65, 0.69, 0.87, 0.97	3.79, 1.09, 38.29, 43.87, 7.82, 4.42
D1	254	14	0.07, 0.11, 0.30, 0.42, 0.44, 0.50, 0.61, 0.64, 0.68, 0.76, 0.81, 0.86, 0.91, 0.96	4.75, 2.50, 3.19, 0.25, 0.17, 3.61, 5.78, 9.39, 15.59, 27.09, 11.16, 5.50, 5.21, 5.81
	366	14	0.06, 0.12, 0.19, 0.30, 0.39, 0.43, 0.50, 0.61, 0.64, 0.68, 0.75, 0.81, 0.86, 0.97	0.43, 1.24, 0.85, 14.48, 0.38, 1.53, 3.10, 6.78, 6.32, 16.62, 16.99, 20.99, 7.87, 2.42
D2	254	11	0.44, 0.48, 0.50, 0.56, 0.64, 0.69, 0.75, 0.80, 0.88, 0.93, 0.96	0.59, 3.04, 1.55, 3.61, 23.81, 40.33, 13.15, 6.04, 3.16, 3.11, 1.62
	366	8	0.38, 0.48, 0.56, 0.64, 0.68, 0.80, 0.88, 0.93	4.52, 2.02, 6.24, 24.60, 44.55, 12.69, 3.98, 1.41
E	254	8	0.34, 0.40, 0.49, 0.72, 0.75, 0.85, 0.92, 0.96	0.42, 1.78, 1.35, 31.66, 31.85, 21.95, 5.35, 5.63
	366	4	0.69, 0.75, 0.80, 0.96	32.52, 14.00, 48.15, 5.33
P	254	3	0.71, 0.86, 0.93	2.26, 88.63, 9.10
	366	3	0.66, 0.86, 0.95	1.80, 96.62, 1.58

A - A method, B - B methods (3 fraction), C - C method, D - D methods (2 fraction), E - E method, (P) (piperine) control, (254 and 366) wavelength at 254 and 366 nm. TLC: Thin layer chromatography

Calculation of total alkaloid

Calculation of total alkaloid used an indirect way because that measured is bismuth bounded on the alkaloids, instead of alkaloid compound. The Dragendorff reagent additions caused the formation of precipitate complexes between alkaloids and bismuth compound. The bismuth is irrespective of its ties with the alkaloid with the
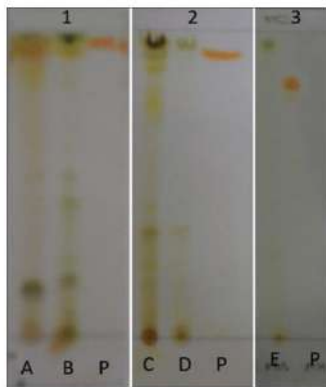
Fig. 2: Identification alkaloid using Dragendorff spray reagent, (1) using dichloromethane:methanol eluent (95:5), (2) using dichloromethane:methanol eluent (97:3), (3) using toluene:acetone:ethanol:25% ammonia (20:20:3:1), (A) Method A, (B) Method B, (C) Method C, (D) Method D, (E) Method E, and piperine (control)
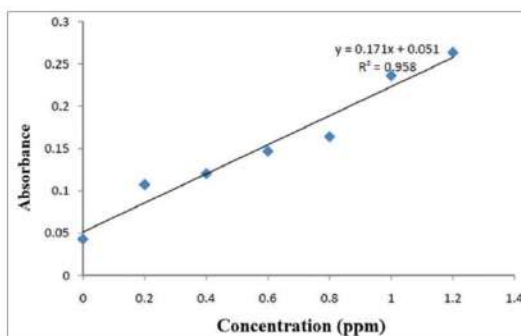

Fig. 3: Calibration curve and linear regression of piperine as a standard

Table 4: Absorbance of total alkaloid test results

Concentration (ppm)	Absorbance	Linear regression formula
DCM fraction (117 ppm)	0.1990	Y=0.171X+0.051 with r=0.958
Piperine (standard)		
0	0.0430	
0.2	0.1075	
0.4	0.1205	
0.6	0.1470	
0.8	0.1640	
1.0	0.2360	
1.2	0.2635	

DCM: Dichloromethane

addition of sodium sulfide. The addition of thiourea in the medium led to the formation of nitric acid complex $Bi[CS(NH_2)_3]_3(NO_3)_3$ in the form of a yellow precipitate. The precipitate measured with visible light 435 nm [22].

The selected standard as a Piperine is an alkaloid compound derived from the family of Piperaceae which is expected to describe the alkaloids contained in *P. pellucida*. The piperine as a standard was made in several concentrations (including 0, 0.2, 0.4, 0.6, 0.8, 1, and 1.2 ppm), and the

absorbances were measured using spectrophotometer at wavelength 435 nm (Table 4 and Fig. 3). Total alkaloids in DCM fraction amounted to 29.59 mg/g piperine.

CONCLUSION

Based on the results of the study of the extraction method development with the alkaloid enrichment purposes obtained TLC profiles of different uses three kinds of eluent combinations of separating spots of the compound contained in each extract, these results will be the basis for isolation of alkaloids. While for identification still needs to be done using the special way that can be detected alkaloid compounds in the plants.

ACKNOWLEDGMENTS

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