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IN VITRO INVESTIGATION OF ANTDIARRHOEAL, ANTIMICROBIAL AND THROMBOLYTIC ACTIVITIES OF AERIAL PARTS OF *PEPEROMIA PELLUCIDA*

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Abstract

Peperomia pellucida is a medicinal herb belonging to the family of Piperaceae-Pepper. It is also known as Luchi Pata in Bangladesh and has a history of ethno-medicinal properties. The crude ethanolic extract of *Peperomia pellucida* leaves was partitioned successively by solvents of different polarity. All fractions were then investigated for qualitative preliminary phytochemical screening as per standard procedure. The various fractionates of the leaves of *P. pellucida* were designed to evaluate for their possible antimicrobial, thrombolytic and antidiarrhoeal activities by using standard drugs; Penicillin for antimicrobial, streptokinase (SK) for thrombolytic and Loperamide for antidiarrhoeal activities. In this study, ethyl soluble fractions (ESF) showed maximum activity of 50.65% against thrombosis in comparison with (SK) 65%. In the antidiarrhoeal assay the extract (ESF) inhibited the mean number of defecation by 41.81% and 60.18% ($p<0.01$ & $p<0.001$) at 250 mg/kg and 500 mg/kg body weight, respectively. The latent period for the extract treated group was ($p<0.01$ & $p<0.001$) increased as compared to control group The extracts showed varying degree of inhibitory potential against all the tested bacteria. Ethanol extract of the plant had higher inhibitory action against *Staphylococcus aureus, Bacillus subtilis*, *Proteus mirabilis* and *Pseudomonas fluroescens*. The results of the present study revealed that the leaves of this traditional medicinal plant are a remedy for thrombosis, diarrhoea and possess potential antimicrobial activity against important human pathogens.

Key words: *Peperomia pellucida*, streptokinase, loperamide, penicillin, thrombolytic activity, antidiarrhoeal activity.

Introduction

Medicinal plants are one of the important contributors to most of the medicinal preparations as raw plant materials, refined crude extracts and mixtures etc. Several thousands of plants have been identified containing medicinal values and are used to treat different ailments in various cultures worldwide. Even in this modern world, majority of the people are still relying on the traditional medicine for their primary health care. [1] *P. pellucida* is a well-known plant in Bangladesh which is used in different as a potential medicinal plant against various ailment, this plant *(*Family: Piperaceae-Pepper) grows well in along roadsides, in plantations, on damp ground in shady places near houses from sea level to 400 m elevation. It has different common names in different languages e.g. Luchi Pata. [Bengali], little heart. [Brazil], Varshabhoo [Sanskrit], Pepper elder. [English], Suna kosho. [Japan]. The plant is glabrous and its roots are fibrous, stems are translucent pale green, erect or ascending, usually 15-45 cm long, internodes usually 3-8 cm long. The leaves are medium green on upper surface, and whitish green on the lower surface. Its traditional use in Southeast Asia includes: in curing the wounds. Its crushed mixture with water is used to cure hemorrhage. People of Java used it to control abdominal pain. Malays used this plant for the treatment of fatigue and rheumatism. In Bangladesh, the juice of the leaves is used for reliving cough and fever. It is also used as remedy for colds, diarrhea, heart problems, and hypertension [2]. In recent times, the research activities have been focused on the phytochemical investigation of the plants which have ethno-botanical and folkloric importance associated with them for drug discovery [3,4]. Because of its copious, widespread availability and folkloric use, the present study was undertaken to investigate the potential of *Peperomia pellucida* leaves extract as antidiarrhoeal, thrombolytic agent and antimicrobial activity against important human pathogens.

Materials and methods

Plant materials

The plant, *Peperomia pellucida* was collected from the National Botanical Garden, Dhaka, Bangladesh in May, 2014 and was identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka. Voucher specimens for this plant have been maintained in Bangladesh National Herbarium for future reference.

Preparation of extract

The collected plant leaves were shade dried for several days and then oven dried for 24 hours at 40 °C to facilitate grinding. The powdered leaves were then stored in a tight container. The dried powder material (400 gm) was soaked in 1500 ml of 90% ethanol for ten days and was shaken occasionally. The whole mixture was filtered by a piece of clean, white cotton. The filtrate so obtained was filtered again through Whatman filter paper. The filtrate was concentrated using a vacuum rotary evaporator at optimum temperature of 40-50 °C to obtain the crude extract of *P. pellucida.* The concentrated aqueous ethanol extract was partitioned by the Kupchan method and the resultant partitionates, i.e. Chloroform, ethyl acetate, hexane and aqueous soluble materials were used for the current investigation.

Preliminary Phytochemical screening

The freshly prepared organic extracts were qualitatively tested for the presence of various classes of Phytochemicals. These were identified by characteristic color changes using standard procedures [5]. Results of preliminary phytochemical testing showed the presence of major classes of phytochemicals such as carbohydrates, steroids, alkaloids, tannins and flavonoids in the extracts (Table 1). Hexane soluble fraction (HXSF) gave color reactions for all secondary metabolites except carbohydrates. Though, CSF and ESF showed the presence of non reducing carbohydrates but steroids were absent in both the extracts.

Determination of total tannin content.

The tannins were determined using the Folin-coicalteu phenol reagent as reported by Amorim. [6] Briefly, 0.1 ml of the sample extract was added to 7.5 ml of distilled water and 0.5 ml of Folin-coicalteu phenol reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of tannic acid is read against a blank. The results of tannins are expressed in terms of tannic acid in mg/g of extract. Total tannin content was determined as mg of tannic acid equivalent per gram using the equation obtained from a standard tannic acid calibration curve with intercept (C) = -0.2583, slope (m) = 4.5692 and regression co-efficient R2 = 0.9953.

Statistical analysis.
Data were presented as mean ± standard error mean (SEM). Statistical analysis was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. P values < 0.05 were considered to be statistically significant (*p* indicates probability)

Determination of total flavonoid content
The total flavonoid content was estimated using a method previously described by Kumaran and Karunakaran using quercetin as a reference compound. Aluminum chloride colorimetric method was used to determine the total flavonoids content in the plant extract of different partitionates of the *P. pellucida.* This method is based on the determination of the flavonoid-aluminum complex between flavonoid of the crude extract and aluminum chloride. Briefly, 1 ml of leaves extracts in methanol (50-250 µg/ml) was mixed with 1 ml aluminum chloride in ethanol (20 mg/ml) and a drop of acetic acid. The resulting mixture was then diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min. A blank sample was prepared in similar fashion omitting the extract. The calibration curve of quercetin was plotted using the same procedure and the amount of total flavonoids was calculated from linear regression equation obtained from the curve (y = 0.0098x - 0.0364; R^2 = 0.9724) and expressed as quercetin equivalents (QAE) per gram of the plant extract.

Test for antidiarrhoeal activity
Test animals & drugs
White albino mice (Swiss-wistar strain, body weight: 20-25 gm) of both sexes were used for *in vivo* antidiarrhoeal activity. They were housed in standard environmental conditions at animal house of Pharmacology Laboratory, World University of Bangladesh, Dhaka, Bangladesh. Animals were kept under standard environmental conditions (temperature: (24.0 ± 1.0 ˚C), relative humidity: 55-65% and 12 hr light/12 hr dark cycle) and had free access to food and water. The cages were cleaned once daily. This study was carried out following approval from the ethical committee comprising pharmacologist and toxicologist expert on the use and care of animals of the BCSIR. Loperamide (Square Pharmaceuticals Ltd., Bangladesh) was used as standard drug for this study

Chemicals
Folin- coicalteu phenol reagent and tannic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tween 80 and sodium carbonate were of analytical grade and purchased from Merck (Darmstat, Germany).

Castor oil-induced diarrhoea
Antidiarrhoeal activity of leaf extract of *P. pellucida* was tested by using castor oil induced method in mice [7,8]. Twenty swiss albino mice were randomly divided in to four groups (n = 5). Control group received only distilled water 2 ml/mice, positive control group received loperamide 50 mg/kg body weight as standard and test groups received the extracts at the doses of 250 mg and 500 mg/kg body weight. Mice were housed in separate cages having paper placed below for collection of fecal matters. Diarrhea was induced in the mice by oral administration of castor oil (1.0 ml/mice). Extract and drugs were given orally 1 hour before the administration of castor oil. The time for first excretion of feces and the total number of fecal output by the animals were recorded. Normal stool was considered as numerical value 1 and watery stool as numerical value 2. Percent inhibition of defecation in mice was calculated by using the following equation:

$$\% \text{ inhibition} = [(Mo–M)/Mo] \times 100;$$

Where: Mo = Mean defecation of control and M = Mean defecation of test sample.

In vitro thrombolytic activity
Sample preparation
The crude extracts were suspended in 10 ml of sterile distilled water and shaken vigorously on a sonicator. The suspension was kept overnight and decanted to remove soluble supernatant, which was filtered through a filter paper. The clear solution so obtained was used directly for *in vitro* evaluation of clot lysis activity**.**

Thrombolytic assay
The thrombolytic activity of prepared extracts was evaluated by the method of Daginawala, using streptokinase (SK) as a standard drug**.** Whole blood (5 ml) were drawn from healthy volunteers, and transferred in different pre-weighed sterile tubes (1 ml/tube) to form clots and incubated at 37 ˚C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot formed and each tube having clot, was again weighed to determine the clot weight [clot weight = weight of clot containing tube – weight of tube

alone]. The tubes containing pre-weighed clot was properly labeled, 100 µl crude extract and aqueous solutions of different fractionates were added to the tubes separately. As a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of isotonic solution was separately added to the clot containing tubes. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and vials were again weighed to observe the difference in weight after clot disruption. Differences obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt. of released clot /clot wt.) × 100.

Antimicrobial activity

Test bacteria

A total of eight bacterial strains were used in the present study. Three Gram positive bacterial species viz. *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* and five Gram negative strains such as *Escherichia coli, Proteus mirabilis, Pseudomonas fluroescens*, *Pseudomonas aeruginosa* and *Salmonella typhi* were used to evaluate the antibacterial activity of the leaves extracts. These pathogenic strains were obtained from the Department of Microbiology, University of Dhaka. The bacterial strains were maintained on nutrient agar slants at 4 °C.

Antibacterial activity

The extracts obtained were screened for their antibacterial activity in comparison with standard antibiotic penicillin (10 mg/mL) *in vitro* by disc diffusion method using various bacterial strains [14]. The paper discs (6 mm diameter, Whatman No. 1 filter paper) containing 1.0, 2.0, 5.0, 10.0 mg/ml plant extracts were dried and placed aseptically on the agar surface with the help of a sterile forceps and paper discs were pressed slightly with the forceps to make complete contact with the surface of the medium [9]. The plates were kept at room temperature for half an hour and subsequently incubated at 37 °C and observed for zone of inhibition after 24 hours. The assay was carried out in triplicate and the inhibition zone around each disc was measured in millimeter. The results were recorded by measuring the zone of growth inhibition surrounding the disc.

Data analysis

The results are expressed as mean ± SD. Student's paired t-test was used to analyze level of statistical significance between groups. *P*-value less than 0.01 was considered statistically significant**.**

Results and Discussion

Test for antidiarrhoeal activity

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the faeces. In some diarrheas, the secretory component predominants, while other diarrhoeas are characterized by hypermotility. The use of castor oil induced diarrhoea model in our study is logical because the autacoids and prostaglandins are involved in producing diarrhoea in human. The liberation of ricinolic acid from castor oil results in irritation and inflammations of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [10]. These observations suggest that those extracts at a dose of 250 mg/kg & 500 mg/kg reduced diarrhoea by inhibiting castor oil induced intestinal accumulation of fluid. Phytochemical screening revealed the presence of reducing sugar, tannin, steroid & alkaloid. Earlier studies showed that anti-dysenteric and antidiarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids and sterols [11, 12]. gain, tannins and phenolics present in the plant extract are reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil. Hence, tannins, steroid & alkaloid may be responsible for anti-diarrhoeal activity of *P. pellucida* leaves.The result showed that the extract reduced the mean number of defecation which were 41.81% and 60.18% ($P<0.01$ & $P<0.001$) at the doses of 250 mg/kg and 500 mg/kg respectively. The latent period for the extract treated group was ($P<0.01$ & $P<0.001$) increased as compared to control group and are presented in table 2.

Total flavonoid content

Flavonoids are widely distributed in plants, fulfilling many functions. It has been recognized that flavonoids presence show anti-inflammatory activity and their effects on human nutrition and health are of paramount importance. Preliminary studies indicate that flavonoids may affect anti-inflammatory mechanisms via their ability to inhibit reactive oxygen or nitrogen compounds. Flavonoids have also been proposed to inhibit the pro-inflammatory

activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase and to modify intracellular signaling pathways in immune cells, or in brain cells after a stroke. Total flavonoid contents of various fractions of *P. pellucida* leaves extracts were expressed as mg of quercetin equivalents/gm of dried extract and are presented in table 3. Since all the fractions exhibited positive results for flavonoid in the preliminary phytochemical screening, the same is quantified by the analysis of total flavonoid content. However, ethyl soluble fraction exhibited the highest flavonoid content (152.17 ± 0.15 mg/g) while hexane soluble fraction was found to contain the lowest flavonoid content (41.47 ± 0.47 mg/g)

Total tannin content

Phytochemical screening revealed the presence of tannin, steroid & alkaloid. Earlier studies showed that anti-dysenteric and antidiarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids and sterols [13]. Again, tannins and phenolics present in the plant extract are reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil [14]. Tannins, steroid & alkaloid may be responsible for anti-diarrhoeal activity of *P. pellucida* leaves. The total tannin content was calculated as quite high in ethanolic crude extract (251.41 mg/g of tannic acid equivalent) and are presented in table 4.

Thrombolytic activity

Thrombosis is the formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system. When a blood vessel is injured, the body uses platelets (thrombocytes) and fibrin to form a blood clot to prevent blood loss. Even when a blood vessel is not injured, blood clots may form in the body under certain conditions [15]. Several thrombolytic drugs obtained from various sources are used for the treatment of thrombosis. Thrombolytic agents are used to disrupt already formed blood clots in clinical settings where ischemia may be fatal (acute myocardial infarction, pulmonary embolism, ischemic stroke, and arterial thrombosis). Thrombolytic drugs dissolve blood clots by activating plasminogen, which forms a cleaved product called plasmin. Plasmin is a proteolytic enzyme that is capable of breaking cross-links between fibrin molecules and can provide the structural integrity of blood clots. Because of these actions, thrombolytic drugs are also called plasminogen activators and fibrinolytic drugs. There are three major classes of fibrinolytic drugs: tissue plasminogen activator (tPA), streptokinase (SK), and urokinase (UK). These drugs have the ability to effectively dissolve blood clots [17]. The results of *in vitro* thrombolytic activity study revealed that addition of 100 µl streptokinase (SK) for fibrinolytic drugs as a positive control (30,000IU) to the clots showed 62.67% lysis of clot on the other hand sterile distilled water a, negative control exhibited a negligible percentage of lysis of clot 3.23%. The *in vitro* thrombolytic activity of various extracts was found in the range of 10.87-55.56%. The ethanolic soluble fraction (ESF) showed 50.65% lysis of clot, hexane soluble fraction (HXSF)- 44.71%, chloroform extract (CSF) 27.71%, and aqueous soluble fraction (AQSF) 22.81% , respectively. The ethanolic soluble fraction (ESF) of *P. pellucida* exhibited highest thrombolytic activity comparable to standard drug, SK. The significance between percentages of clot lysis of crude extract by means of weight difference was tested by the paired t-test analysis. Statistical representation of the effective clot lysis percentage by different fractionate of crude leave extract results were compared with positive thrombolytic control (streptokinase) and negative control (sterile water), p values < 0.01 was considered statistically significant, the results have been shown in table 5. The thrombolytic activities of the extract was found in the following order ESF>HXSF> CSF>AQSF.

Antibacterial activity

The antibacterial activity of the ethanolic extract of leaf of *P. pellucida* was studied against both gram positive microbes, *Staphylococcus aureus, Bacillus subtilis* and *Bacillus cereus* and gram negative species, *Escherichia coli, Proteus mirabilis*, *Pseudomonas fluroescens*, *Pseudomonas aeruginosa* and *Salmonella typhi* at 4 different concentrations (1.0, 2.0, 5.0 and 10.0 mg/ml) and the antibacterial activity was compared with the standard antibiotic penicillin (10 mg/mL). The results of antibacterial screening of hexane, chloroform, ethyl acetate, ethanol, and water extracts of *P. pellucida* are presented in table 6 and 7. The results revealed variability in inhibitory concentrations of each extract against a given bacteria. The inhibition of bacterial growth was dose dependent since the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains as evident by the higher zone of inhibitions at higher concentrations of each extract. Among the various extracts used, hexane extracts of *P. pellucida* showed

the highest activity (zone of inhibition 18.4 mm) against *Proteus mirabilis*. Hexane extract was appeared to be the most effective extract. None of the water extracts showed any antibacterial activity. Chloroform extracts was inactive against all the gram positive bacteria tested. The antibacterial activity of the extracts was more prominent on the gram negative bacteria than the gram positive bacteria. The antibacterial activity of the plants may be due to the presence of various active principles in them. Plant extracts often contains polyphenols and flavonoids which could be the antimicrobial components [16]. The bioactivity of plants extracts is attributed to phytochemical constituents. Flavonoids are a major group of phenolic compounds reported for their antiviral, antimicrobial and spasmolytic properties. Alkaloids isolated from plants are commonly found to have antimicrobial properties. The antibacterial activities of these compounds might be due to their ability to complex with bacterial cell wall and therefore, inhibiting the microbial growth.

Conclusion

The extracts of different polarities of *P. pellucida* showed significant *in vitro* antimicrobial, thrombolytic and antidiarrhoeal activities. It may be assumed that these extracts can be considered as good source of antimicrobial, thrombolytic and antidiarrhoeal agents. More detailed phytochemical analysis will be necessary to isolate and characterize the active compounds responsible for the thrombolytic, and antidiarrhoeal activities as well as to understand the exact mechanisms of action of these activities. The present study also justifies the claimed uses of *P. pellucida* in the traditional system of medicine to treat various infectious diseases caused by the microbes.

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Table 1. Results of phytochemical screening of different partitionates of *P. pellucida.*

Test for	HXSF	CSF	ESF
Carbohydrates	-	+	+
Steroid	+	-	-
Reducing sugar	-	-	-
Alkaloids	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+

+ Indicate present; - Indicate absent

Table 2. Antidiarrhoeal activity of the ethanolic extract of leaves of *P. pellucida* in castor oil induced diarrheal test method on mice.

		Mean± SE		
Sample	**Dose**	**Latent period**	**Defecation**	**% Inhibition**
Distilled water	2 ml/mice p.o	0.79 ± 0.06	9.9 ± 0.86	_
Loperamide	50mg/kg p.o	2.21 ± 0.16**	3.9 ± 0.45**	70.38
Ethanolic extract of *P. pellucida*	250 mg/kg p.o	1.05 ± 0.07*	5 .9 ± 0.86	41.81
	500 mg/kg	1.59 ± 0.19**	4.9 ± 0.63**	60.18

Values are expressed as mean ± SEM (Standard Error Mean); * indicates P < 0.01; ** indicates P < 0.001, one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o: per oral

Table 3. Total flavonoids contents of the ethanolic extracts of *P. pellucida.*

Different fractionates of leave extract	Total flavonoids mg/g plant extract (in QAE)
ESF	152.17 ± 0.15
HXSF	41.47 ± 0.47
CSF	60.76 ± 1.80

Results are expressed as mean ± SD

Table 4. Total tannin content of ethanol extract of *P. pellucida* leaves

Extract	Average absorbance at 725 nm	Total tannin content mg of tannic acid equivalent (TAE)per gm of dry extract
Ethanol extract of *P. pellucida*	0.92 ± 0.19	249.41 ± 0.88

Values are expressed as mean ± SEM (Standard error mean).

Table 5. Thrombolytic activity of different fractionates crude extract of *P. pellucida.*

Fractions	Weight of empty vial (A) g	Weight of vial with clot (B) g	Weight of clot (B-A)g	Weight of vial with clot after lysis (D) g	Weight of clot lysis (B-D)g	% of clot lysis
ESF	5.14	5.91	0.77	5.52	0.39	50.65*
HXSF	5.29	6.14	0.85	5.76	0.38	44.71*
CSF	5.28	5.75	0.47	5.62	0.13	27.66*
Blank	5.20	5.73	0.53	5.70	0.03	5*
SK	4.65	5.05	0.4	4.79	0.14	65*
AQSF	5.47	6.04	0.57	5.91	0.13	22.81*

*P<0.01, crude extracts are significant as compared to positive control and negative control

Table 6. Antibacterial activity of extracts of *P. pellucida against* Gram positive bacteria.

Extract	Concentration of extract (mg/mL/disc	Zone of inhibition (mm) Gram positive bacteria		
		Staphylococcus aureus	***Bacillus subtilis***	***Bacillus cereus***
Ethyl acetate	1	8.4 ± 0.05	_	_
	2	12.2 ± 0.17	_	_
	5	13.5 ± 0.13	12.4 ± 0.15	_
	10	14.5 ± 0.16	13.8 ± 0.29	_
Chloroform	1	_	_	_
	2	_	_	_
	5	_	_	_
	10	_	_	_
Ethanol	1	10.5 ± 0.37	_	_
	2	11.7 ± 0.39	_	_
	5	13.8 ± 0.02	10.7 ± 0.25	9.5 ± 0.48
	10	16.3 ± 0.18	11.5 ± 0.22	9.8 ± 0.31
Aqueous	1	_	_	_
	2	_	_	_
	5	_	_	_
	10	_	_	_
***n*-Hexane**	1	_	8.4 ± 0.043	_
	2	_	11.2 ± 0.39	_
	5	8.8 ± 0.03	12.3 ± 0.67	8.5 ± 0.48
	10	9.7 ± 0.02	17.9 ± 0.26	9.7 ± 0.33

The results are mean ± SD (n=8)

Table 7. Antibacterial activity of extracts of *P. pellucida* against Gram negative bacteria.

Extract	Concentration of extract (mg/mL/disc)	Zone of inhibition (mm) Gram positive bacteria				
		Escherichia coli	***Proteus mirabilis***	***Pseudomonas fluroescens***	***Pseudomonas aeruginosa***	***Salmonella typhi***
Ethyl acetate	1	8.2 ± 0.54	_	_	_	_
	2	10.2 ± 0.43	_	_	_	_
	5	12.0 ± 0.79	13.2 ± 0.35	9.4 ± 0.59	8.1 ± 0.33	7.9 ± 0.84
	10	16.2 ± 0.24	14.6 ± 0.34	13.3 ± 0.36	7.9 ± 0.24	7.2 ± 0.64
Chloroform	1	_	_	7.9 ± 0.17	_	_
	2	_	_	9.3 ± 0.38	_	_
	5	9.3 ± 0.18	_	11.7 ± 0.36	8.1 ± 0.33	_
	10	12.3 ± 0.57		14.9 ± 0.29	8.8 ± 0.24	8.2 ± 0.64
Ethanol	1	7.3 ± 0.49	8.4 ± 0.22	7.3 ± 0.24	7.2 ± 0.32	_
	2	9.4 ± 0.18	10.5 ± 0.55	9.4 ± 0.44	8.2 ± 0.52	_
	5	12.3 ± 0.31	13.2 ± 0.75	11.6 ± 0.66	8.6 ± 0.22	7.2 ± 0.26
	10	14.0 ± 0.22	16.2 ± 0.82	14.5 ± 0.29	10.6 ± 0.12	7.8 ± 0.14
Aqueous	1	_	_	_	_	_
	2	_	_	_	_	_
	5	_	_	_	_	_
	10	_	_	_	_	_
***n*-Hexane**	1	3 ± 11.09	9.3 ± 0.12	7.1 ± 0.68	10.4 ± 0.49	7.6 ± 0.75
	2	6 ±11.09	11.3 ± 0.32	8.6 ± 0.17	10.3 ± 0.39	7.9 ± 0.38
	5	8.2 ± 0.66	15.3 ± 0.33	11.3 ± 0.19	9.8 ± 0.58	8.4 ± 0.52
	10	12.1 ± 0.78	18.4 ±0.35	15.5 ± 0.33	11.9 ± 0.73	8.8 ± 0.69

The results are mean ± SD (n=8)