Analysis of Cytotoxic Activity of Some Folkloric Medicinal Plants as Used for Cough Treatment

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Abstract

In this paper highlights the preliminary phytochemical constituents of three selected medicinal plants and also provides free from cytotoxic usages of folkloric medicinal plants. In research concerns with the analysis of cytotoxic activity of three folkloric medicinal plants which are used as cough treatment-*Polygonum chinense* L. (**Mahagar-kyansit),** *Scoparia dulcis* L. (**Danna-thuka)** and *Ludwigia octovalvis* (Jacq.) Raven **(Lay-nhyin-gyi**) in Rakhine state. These selected plants are small perennial herbs. The whole plant of all selected plants were separately used for all tested experiments. Cytotoxicity of aqueous and ethanol extracts of all selected plants were determined by brine shrimp lethality bioassay. In that experiment, potassium permanganate and caffeine were used for positive control. The medium lethal concentration (LC_{50}) values of cytotoxicity tests were calculated based on probit values and log.concentration of tested extracts by excel linear regressing program. As the LC_{50} value of ethanol extract of *Ludwigia octovalvis* is 482.39 μgmL^{-1}, it may be considered that extract is medium toxic by Clarkson's toxicity criteria for plant extracts. Other tested extracts have no toxic according to their results of LC_{50} values.

Keywords: folkloric medicinal plants, cytotoxicity, Brine shrimp lethality bioassay, LC_{50}

INTRODUCTION

Animals, including humans depends directly or indirectly on plant derived extracts or products are most important roles for food security, health care, environment, religion, culture, and development. However, some cases animals have obtained the toxicins from plants, example; the monarch butterfly (*Danaus plexippus*) and the poison dart frog (*Dendrobatidae*) found in the rain forests of Central and South America (Culter & Culter, 2000). Most plants contain many chemical constituents that provide to the health benefited compounds. Traditional medicine or folk medicine is based on extracts from plants. In times of illness, herbal remedies were used in various ways to treat ailments by plant extracts. Today, although synthetic drugs are being used, folkloric medicine is still used to cure ailments. According to folkloric medicine, most people in Myanmar are using herbs as a remedy for the treatment of diseases such as cough and respiratory related diseases, toothache, etc. In this paper concerned with three herbal plants such as Mahar-gar-kyan-sit, dan-ta-thuhka and lay-nyin-gyi still using folkloric medicines to cure cough related disease in the rural area of Rakhine state. Aim of this paper is to estimate for safety dosages for ingestion of folkloric medicine for those herbal plants.

The scientific name of Mahagar-kyansit is *Polygonum chinense* L, it belongs to Polygonaceae. It is a perennial flowering shrub and it grows a high 1-1.5 meters. It can be found growing on wastelands and roadsides (Chan, *et al*., 2003). The chemical constituents of *Polygonum chinense* L. are flavonoids, quinones, tannins, terpenoids, saponins, glycosides, alkaloids and steroids which show anticancer, antitumor, anti-oxidative, anti-inflammatory, analgesic, antibacterial, insecticidal, and other pharmacological effects (Tran, *et al*., 2017 and Vaidyaratnam, 1994).

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The scientific name of Danta-thuhka is *Scoparia dulcis* L. It belongs to plantaginaceae and is a perennial erect herb with many branches (Tun, *et al*., 2006). It widely distributed in many tropical countries in the world and is found in abundance in South America and the Amazon rainforest including Myanmar. It contained acacetin, amyrin, apigenin, benzoxazin, benzoxazolin, benzoxazolinone, betulinic acid, cirsimarin, cirsitakaoside, coixol, coumaric acid, cynaroside, daucosterol, dulcinol, dulcioic acid, friedelin, gentisic acid, glutinol, hymenoxin, ifflaionic acid, linarin, luteolin, mannitol, scopadiol, scopadulcic acid A & B, scopadulciol, scopadulin, scoparic acid A to C, scoparinol, scutellarein, scutellarin, sitosterol, stigmasterol, taraxerol, vicenin, and vitexin. (Tun, *et al*.,2006). Medicinal uses of *Scoparia dulcis* L. for menstrual problems, for upper respiratory bacterial and viral infections and to relieve pain of various type (headaches, stomachaches, muscle pain, etc.) and for venereal diseases and urinary tract infections. The whole plant of it is mainly used in antimalarial. This medicinal plant is very popular for treatment of dental diseases in Myanmar. (Tun, *et al*., 2006).

The Scientific name of Lay-nhyin-gyi is *Ludwigia octovalvis* (Jacq.) Raven. belongs to onagraceae. It is a herbaceous shrub, it can grow up to 2 m tall and has a branched growth form. It is reported also in Australia, the Pacific Islands, Japan, South and Southeast Asia including Myanmar (Henty and Pritchard, 1975). Thirteen chemical organic compounds were obtained and determined as follows: beta-sitosterol , oleanolic acid , 2α-hydroxy ursolic acid , tormentic acid , daucosterol, maltol), luteolin , quercetin , apigenin , methyl brevifolin carboxylate), gallic acid, 3, 4, 8, 9, 10-pentahydroxydibenzo [b, d] pyran-6-one , and ellagic acid (Jing and Yang, 2006). It is traditionally used to treat skin diseases, diarrhea and flatulence (Haidar, *et al*., 2013).

(a) (b) (c)

Figure 1. Photographs of (a) the whole plants of Mahagar-kyansit, (b) Danta-thuhka and (c) Lay-nyinn-gyi

MATERIALS AND METHODS

Samples collection and preparation

Freshly whole plants of *Polygonum chinense* L. (Mahagar-kyansit) were collected from quarter (4), *Scoparia dulcis* L. (Danta-thuhka) from quarter (3) and *Ludwigia octovalvis Juss.* (Lay-nyin-gyi) from quarter (6), Mingan Village, Sittway township, Rakhine State see in Figure 2 and 3. All plants sample were collected in Junary, 2022.

The scientific name of collected three medicinal plants were confirmed by an authorized botanist at the department of Botany, Sittway University.

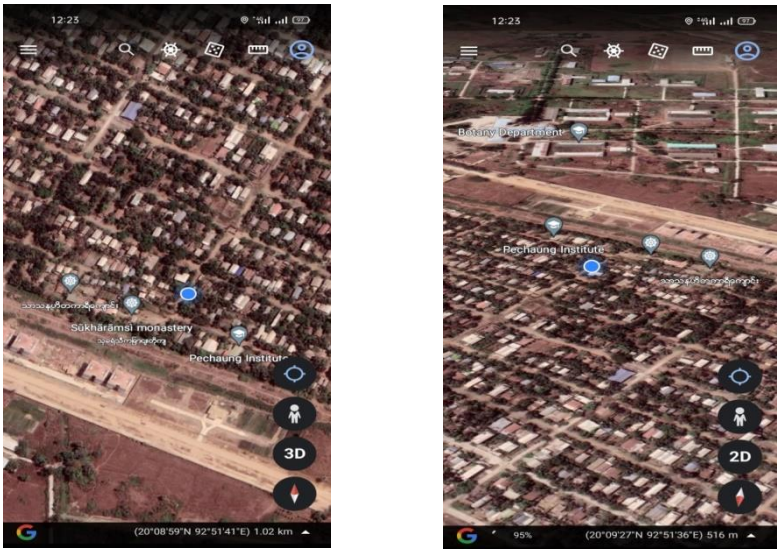
The collected plant samples were separately washed with water and dried under shade at room temperature. The dried samples were cut into tiny pieces and ground to powder by electric grinder. The fine dried powdered samples were stored with air tight bottles.

Chemical requirements

Mayer's reagent, Wagner's reagent, Dragendorff's reagent, ninhydrin reagent, glacial acetic acid, hydrochloric acid, sulphuric acid**,** potassium permanganate, magnesium ribbon, gelatin, acetic anhydride, acetone, lead acetate, potassium iodide, iodine, mercuric chloride, ferric chloride, sodium chloride, caffeine, pet-ether, ethanol, ethyl acetate, chloroform, methanol, distilled water were used for all experiments.

Apparatus requirements

Conical flasks, digital balance (Shimadzu), funnels, filter papers, glass rods, glass tubes, reagent bottles, separating funnel (250 mL), separating funnel (250 mL), stands, pasture pipettes, graduated pipettes, test tubes, water bath, oxygen pump, electric bulb, lens, shaker (TS-2000A VDRL shaker), pH meter (Lovibond, Enso Direct pH 110) were used to achieve the experiments.



(a) (b)

Figure 2. Google map for (a) Polygonam Chinese from quarter 4 and (b) *Scoparia dulcis* from quarter 3, Mingan village, Sittway township

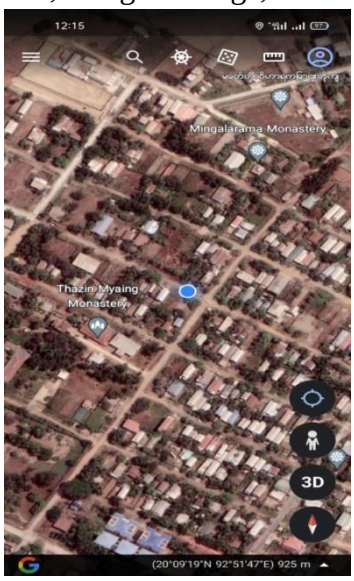


Figure 3. Google map for *Ludwigia octovalvis* from quarter (6), Mingan village, Sittway township

Determination of the Preliminary Phytochemical Constituents

In this experiment, to know the phytochemical constituents, dried powder of whole plant of *Polygonum Chinense, Scoparia dulcis,* and *Ludwigia octovalvis* were carried out simple, standard methods and thin layer chromatographic (TLC) method. In TLC method, pet-ether(PE) and ethyl acetate extracts of selected plants with appropriate eluting solvents systems; PE : EA (90:1, 50:1, 10:1 5:1, and 1:1 v/v).

Preparation of Aqueous extract

Each dried powder sample (30 g) in 500 mL beaker was added 250 mL of distilled water and boiled in water bath for 2 hours. And then it was cooled and filtered. The filtrates were concentrated on water bath at 80℃ and calculated the percentage of amount of extract.

Preparation of Ethanol extract

Each dried powder sample (30 g) in 500 mL beaker was added 250 mL of ethanol and macerated for 24 hours 3 times. After macerating, it was filtered and concentrated on water bath at 80℃. The dried ethanolic extract was obtained and calculated the yield percent of extractive value.

Determination of Cytotoxicity of Three Selected Medicinal Plants

The cytotoxicity of the plant crude extracts was determined by brine shrimp lethality test according to Meyer, 1982 with slightly mortification. Brine shrimp eggs (*Artemia salina* Leach.) were used in that experiment.

Preparation of artificial sea Water

32 g of table salt (without iodine) was dissolved in 1 L of distilled water and filtered off to get clear solution and then was adjusted pH 8.0 by pH meter.

Preparation of test solutions

160 mg of each of crude extracts sample were taken and dissolved in 200 µL of pure dimethyl sulfoxide (DMSO) and finally the volume was made up to 20 mL with artificial sea water. Thus, the concentration of the stock solution was 8000 μgmL^{-1}. Then the solution was serially diluted to 8000.00, 4000.00, 2000.00, 1000.00, 500.00, 250.00, 125.00, 62.50 and 31. 25 μgmL^{-1} with artificial sea water.

Preparation of caffeine solution

32 mg of pure caffeine was taken and dissolved in 200 µL of pure dimethyl sulfoxide (DMSO) and finally the volume was made to 20 mL with sea water. The concentration 3200 μgmL^{-1} of the stock solution was obtained. Then it was serially diluted to obtain 1600, 800, 400, 200, 100, 50 and 25 μgmL^{-1} with artificial sea water.

Preparation of potassium permanganate solution

Potassium permanganate (40 mg) was dissolved in 40 mL of artificial sea water to obtain 1000 μgmL^{-1} concentration. That stock solution was serially diluted to 100.000, 50.000, 25.000, 12.500, 6.250, and 3.125 μgmL^{-1} by artificial sea water.

Hatching brine shrimp for cytotoxicity effect

Brine shrimp eggs (*Artemia salina* Leach.) collected from pet shops were used as the test organisms. 200 mL of artificial sea water was added in the separating funnel and then one tea spoon of brine shrimp eggs was added to one side of the 250 mL of separating funnel and supplied oxygen by air line from the air pump through hatching time with lighted (60 W bulb)

see in Figure 4. After one and half day was allowed to hatch the shrimp and to be matured as nauplii. The hatched shrimps were attracted to the light (phototaxis) and so nauplii free from eggs shell was collected from the illuminated part of the separating funnel. The nauplii were transferred to tested tanks using pasture pipettes.

Brine shrimp lethality bioassay

The cytotoxicity of selected plant crude extracts was determined by Meyer,1985 with slightly modification. In that experiment, by dividing three groups of tests materials were determined. They are (i) positive control groups, (ii) crude plant extracts group, and (iii) negative control group.

(i) In positive control groups were sub-divided by 100.000-3.125 μgmL^{-1} of potassium permanganate solution and 2000.00-31.25 μgmL^{-1} of caffeine solution. In these groups, each 2.5 mL of artificial sea water with 10 nauplii was added to 2.5 mL of each concentration of positive control solutions and stand for 24 hours.

(ii) In crude extracts groups, each 2.5mL of artificial sea water with 10 nauplii was added to 2.5 mL of each selected concentration of tested extracts and stand for 24 hours.

(iii) In negative control group, only 10 nauplii were taken from the fish tank by a pasture pipette and put in 10 mL of fresh clear sea water and stand for 24 hours.

After treating 24 hours, death of nauplii were counted for each of tests for all test groups. All tests were carried out by triplicates. The percent mortality of the tested extracts was calculated according to the total number of deaths nauplii by the following formula;

$$\% \text{ Mortality} = \frac{\text{Number of deaths nauplii}}{\text{Number of dead nauplii + Number of alive nauplii}} \times 100$$

Median lethal concentration (LC_{50}) of tested crude extracts were calculate by constructing the plot with log concentration of tested crude extracts versus probit value. The LC_{50} values were calculated using linear regressive excel program. The cytotoxicity of plant extracts was identified by according to the Clarkson's toxicity Criteria of plants, 2004. LC_{50} value 0-100 μgmL^{-1} is high toxic, LC_{50} value 100-500 μgmL^{-1} is medium toxic, 500-1000 μgmL^{-1} is low toxic and the LC_{50} value above 1000 μgmL^{-1} is no toxic. The percent mortality and LC_{50} values were presented in Table 2 to Table 7.

Figure 4. Hatching brine shrimp

Figure 5. Alive napulii (*Waghulde, et al., 2019)

RESULTS AND DISCUSSION

Determination of Phytochemical Constituents

From results of phytochemical constituents tests: alkaloids, α- amino acids, carbohydrates, cardiac glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, steroids, tannins, terpenoids were observed in selected folkloric medicinal plants such as *Polygonum chinense, Scoparia dulcis and Ludwigia octovalvis*. However, saponins were not observed in *Ludwigia octovalvis*, cholesterol was not found in *Polygonum chinense* and anthocyanins were not present in all three selected plant samples. According to the TLC results, phytochemical constituents such as phenolic compounds, steroids, and terpenoids mainly consisted in all selected medicinal plants were found out. Those findings were consistent with the results of the preliminary phytochemical constituents see in Table 1 and Figure 6, Figure 7 and Figure 8. Those results provide the potential benefits of biologicalical activities of those plants.

Table1. Result of Phytochemical Screening of the Whole Plants of *Polygonum chinense*, *Scoparia dulcis* and *Ludwigia octovalvis*

Test	Extract	Test reagent	Observation		
			Polygonum chinense	***Scoparia dulcis***	***Ludwigia octovalvis***
Alkaloids	1%HCl	Dragendorff's reagent	+	+	+
		Wagner's reagent	+	+	+
		Sodium picrate	+	+	+
Carbohydrates	H_2O	1%α-naphthol	+	+	+
Tannins	H_2O	1% Gelatin	+	+	+
Phenolic	H_2O	Ferric chloride	+	+	+
Glycosides	H_2O	H_2SO_4	+	+	+
Flavonoids	H_2O	10%NaOH, dil.HCl	+	+	+
	EtOH	Mg-ribbon, HCl	+	+	+
α–amino acids	H_2O	Ninhydrin	+	+	+
Terpenoids	$CHCl_3$	H_2SO_4	+	+	+
Saponins	H_2O		+	+	-
Cardiac glycoside	H_2O	Glacial acetic acid, $FeCl_3$, H_2SO_4	+	+	+
Steroids	Acetic acid	$CHCl_3$, H_2SO_4	+	+	+
Cholesterols	$CHCl_3$	H_2SO_4, acetic anhydride	-	+	+
Anthocyanins	H_2O	HCl, NH_3	-	-	-
Reducing sugar	H_2O	Benedict's reagent	+	+	+

(+) present (-) absent (ppt.) precipitate

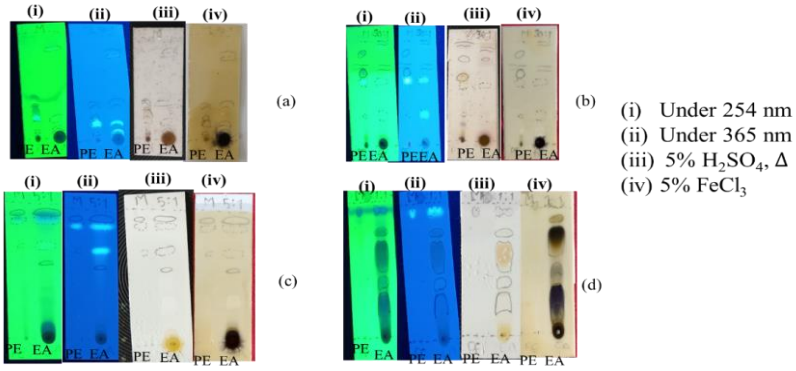


Figure 6. Thin Layer Chromatograms of pet-ether(PE) and ethyl acetate(EA) extracts of *Polygonum chinense* with solvent system PE:EA (v/v); (a) 90:1, (b) 30:1, (c) 5:1, and (d)1:1

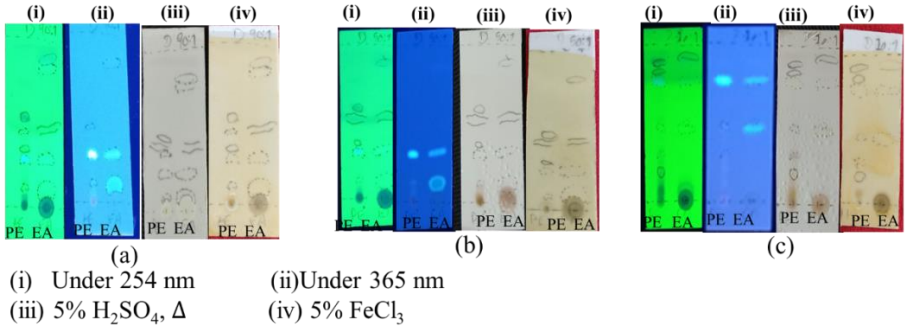


Figure 7. Thin Layer Chromatograms of pet-ether(PE) and ethyl acetate(EA) extracts of *Scoparia dulcis* with solvent system PE: EA (v/v); (a) 90:1, (b) 50:1, and (c)10:1

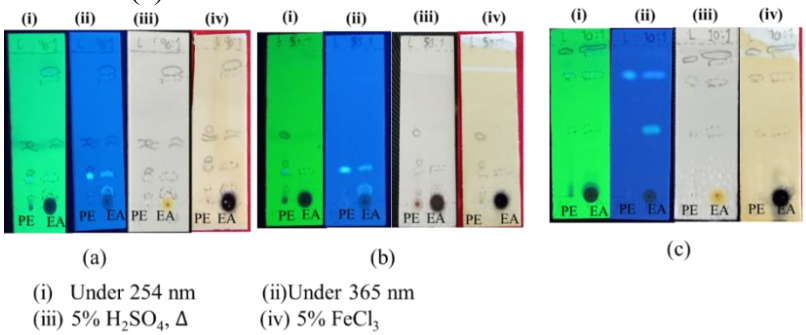


Figure8. Thin Layer Chromatograms of pet-ether(PE) and ethyl acetate(EA) extracts of *Ludwigia octovalvis* with solvent system PE: EA (v/v); (a) 90:1, (b) 30:1, (c) 5:1, and (d) 1:1

Cytotoxicity of Aqueous and Ethanol Extracts of Three Selected Folkloric Medicinal Plants

To conduct the tests for brine shrimp lethality assay of three selected medicinal plants, the crude aqueous and ethanol extracts of *Polygonum chinense, Scoparia dulcis and Ludwigia octovalvis* were obtained (1.26%, 4.6%), (7.06%, 6.9%) and (4.8%, 9.6%). Those extracts were dissolved to obtain the desirable concentration with artificial sea water to check the cytotoxicity of respective plant extracts. The cytotoxicities of those extracts were evaluated in terms of percent mortality and median lethality concentration (LC_{50}) by adding 10 nauplii into three replicates of each concentration of aqueous and ethanol extracts of three selected plants, and after 24 hours the surviving nauplii counting. LC_{50} values were and assessed by

constructing log concentration against probit values according to the Finney table, 1971 using Linear regressing excel program. From the result, the LC_{50} values of the positive controls-potassium permanganate :11.82 μgmL^{-1}; the caffeine: 2026.75 μgmL^{-1}, and tested crude extracts- the aqueous extract of *Polygonum chinense*: 2126.67 μgmL^{-1}; *Scoparia dulcis*: 2327.02 μgmL^{-1}; *Ludwigia octovalvis*: 1860.80 μgmL^{-1}. the ethanol extract of *Polygonum chinense*:1957.49 μgmL^{-1};*Scoparia dulcis*: 2526.97 μgmL^{-1}; *Ludwigia octovalvis*: 482.39 μgmL^{-1}were calculated see in Table 2 to 7.

According to the Clarkson's toxicity Criteria of plants (2004), the aqueous extracts of three selected plants have no toxicity and the ethanol extracts of *Polygonum chinense* and *Scoparia dulcis* have no toxicity, but the ethanol extract of *Ludwigia octovalvis* has medium toxic. Therefore, it may be considered that all three selected medicinal plants may be seemed to be safe if ingestion boiled with water for corrected amount. The ingestion of ethanol soluble contents of *Polygonum chinense* and *Scoparia dulcis* may be seemed to be safe, but *Ludwigia octovalvis* may be needed to be carefully used by ingesting the suitable amount.

Table 2. Percent Mortality and LC_{50} Value of Positive Control, Potassium Permanganate

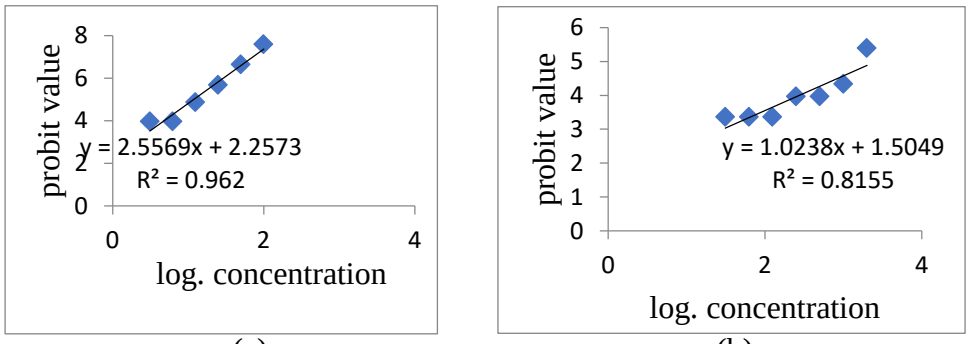
Test	Conc. (μgmL^{-1})	Log conc.	Total no of nauplii survivors	% Mortality	*Probit	LC_{50} (μgmL^{-1})
$KMnO_4$	100.000	2.0000	0	100	7.58	11.82
	50.000	1.6989	1	90	6.64	
	25.000	1.3979	3	70	5.67	
	12.500	1.0969	6	40	4.87	
	6.250	0.7958	9	10	3.96	
	3.125	0.4948	9	10	3.96	

***Finney,1971**

Table 3. Percent Mortality and LC_{50} Value of Positive Control, Caffeine

Test	Conc. (μgmL^{-1})	Log conc.	Total no of nauplii survivors	% Mortality	*Probit	LC_{50} (μgmL^{-1})
Caffeine	2000.00	3.3010	4	60	5.39	2026.75
	1000.00	3.0000	8	20	4.33	
	500.00	2.6989	9	10	3.96	
	250.00	2.3979	9	10	3.96	
	125.00	2.0969	10	0	3.36	
	62.50	1.7958	10	0	3.36	
	31.25	1.4948	10	0	3.36	

*Finney,1971



(a) (b)

Figure 9. Plot of probit value versus log.concentration of (a) $KMnO_4$, and (b) caffeine

Table 4. Percent Mortality and LC_{50} Values of Aqueous and Ethanol Extracts of *Polygonum chinense*

Test	Conc. (μgmL^{-1})	Log conc.	Total no of nauplii survivors	% Mortality	*Probit	LC_{50} (μgmL^{-1})
Aqueous	8000	3.9030	0	100	7.58	2126.67
	4000	3.6020	0	100	7.58	
	2000	3.3010	10	0	3.36	
	1000	3.0000	9	10	3.96	
	500	2.6989	9	10	3.96	
	250	2.3979	10	0	3.36	
Ethanol	8000	3.9030	0	100	7.58	1957.49
	4000	3.6020	0	100	7.58	
	2000	3.3010	9	10	3.96	
	1000	3.0000	9	10	3.96	
	500	2.6989	9	10	3.96	
	250	2.3979	9	10	3.96	

Table 5. Percent Mortality and LC_{50} Values of Aqueous and Ethanol Extracts of *Scoparia dulcis*

Test	Conc. (μgmL^{-1})	Log conc.	Total no of nauplii survivors	% Mortality	*Probit	LC_{50} (μgmL^{-1})
Aqueous	8000	3.9030	0	100	7.58	2327.02
	4000	3.6020	5	50	5.13	
	2000	3.3010	7	30	4.61	
	1000	3.0000	9	10	3.96	
	500	2.6989	10	0	3.36	
	250	2.3979	10	0	3.36	
Ethanol	8000	3.9030	-	100	7.58	2526.97
	4000	3.6020	6	40	4.87	
	2000	3.3010	9	20	4.33	
	1000	3.0000	9	10	3.96	
	500	2.6989	9	10	3.96	
	250	2.3979	9	10	3.96	

Table 6. Percent Mortality and LC_{50} Value of Aqueous Extract of *Ludwigia octovalvis*

Test	Conc. (μgmL^{-1})	Log conc.	Total no of nauplii survivors	% Mortality	*Probit	LC_{50} (μgmL^{-1})
Aqueous	8000	3.9030	0	100	7.58	1860.80
	4000	3.6020	0	100	7.58	
	2000	3.3010	5	50	5.13	
	1000	3.0000	10	0	3.36	
	500	2.6989	10	0	3.36	
	250	2.3979	10	0	3.36	

Table 7. Percent Mortality and LC_{50} Value of Ethanol Extract of *Ludwigia octovalvis*

Test	Conc. (μgmL^{-1})	Log conc.	Total no of nauplii survivors	% Mortality	*Probit	LC_{50} (μgmL^{-1})
Ethanol	2000.00	3.3010	0	100	7.58	482.39
	1000.00	3.0000	2	80	6.04	
	500.00	2.6989	8	20	4.33	
	250.00	2.3979	9	10	3.96	
	125.00	2.0969	10	0	3.36	
	62.50	1.7958	10	0	3.36	
	31.25	1.4948	10	0	3.36	

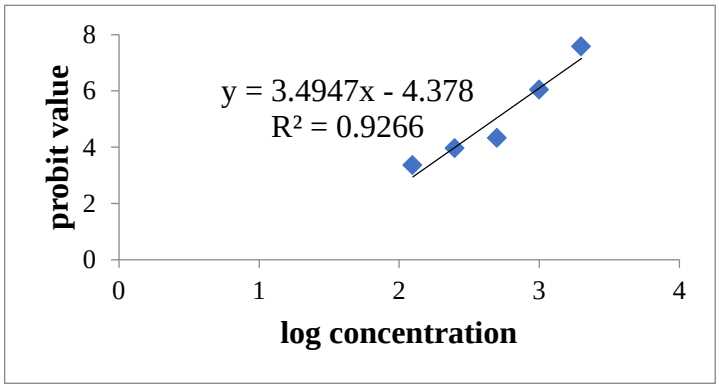


Figure 10. Plot of probit value versus log. concentration of ethanol extract of *Ludwigia octovalvis*

CONCLUSION

According to the results of phytochemical constitutents tests, the mainly important secondary metabolites alkaloids, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, steroids, tannins, and terpenoids are present in three selected medicinal plants. Cytotoxicity of plant extracts are determined based on their LC_{50} values. As the LC_{50} value of ethanol extract of *Ludwigia octovalvis* (482.39 μgmL^{-1}) is less than 500 μgmL^{-1}, it may be seemed medium toxic. Moreover, LC_{50} values of the ethanol extract of *Polygonum chinense,* ethanol extract of *Scopiaria dulcis* and aqueous extracts of three selected plants are greater than 2000 μgmL^{-1}, these plant extracts have no toxic. By compilation of data, except for ethanol extract of *Ludwigia octovalvis*, other tested extracts are free from cytotoxic. Finally, the intake of the free from cytotoxic of selected medicinal plants will be addressed for use of folkloric medicines in rural areas.

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