INVESTIGATION ON ANTIDIARRHOEAL ACTIVITY OF SOME PHYTOCONSTITUENTS OF *Gardenia coronaria* BUCH-HAM. (YIN-GAT-GYI)

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Abstract

The present work concerns with the investigation of some bioactive constituents and anti-diarrhoeal activity of flower and bark of *Gardenia coronaria* Buch-Ham. (Yin-gat-gyi). The preliminary phytochemical investigation reveals the presence of α–amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids and terpenoids in flower while observing alkaloids, carbohydrates, glycosides, organic acids, phenolic compounds, saponins, starch, steroids, tannins and terpenoids in barks. Three organic constituents: benzoic acid (compound I) (0.004%, colourless needle shape), kaempferol (compound II) (0.002%, yellow amorphous form, m.pt = 275-280°C) from EtOAc crude extracts of flower and stigmasterol (compound III) (0.0003%, colourless crystals, m.pt = 167-170°C) from EtOAc crude extract of bark of YGG have been isolated by using thin layer chromatography and column chromatographic separation methods. *In vivo* antidiarrhoeal activity of compound I, compound II, 95 % ethanol and aqueous extracts of YGG were carried out by using castor oil–induced mice models. The mean frequency of defecation in four hours was found to be significantly decreased by both extracts, compound I, compound II and standard drug loperamide compared with that of control in castor oil – induced diarrhoeal in mice ($p<0.005$-$p<0.001$). The percent inhibitions of defecation within four hours for 95 % ethanol extract (1g/kg bw), aqueous extract (4g/kg bw), compound I (5mg/kg bw), compound II (6mg/kg bw) and standard drug loperamide (6mg/kg bw) were 74.41 %, 69.61 %, 57.61 %, 84.78 % and 69.61 %, respectively. It was found that the percent inhibitions of defecation for 95 % ethanol extract (1g/kg bw) and compound II (6mg/kg bw) were higher than that of the standard drug loperamide. Aqueous extract (4g/kg bw), 95 % ethanol extract (1g/kg), compound I and compound II were found to be significantly reduced both volumes and weights of the intestinal fluids secretion, comparable to the

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effect of the standard drug loperamide in castor oil induced enteropooling test ($p < 0.05 – p < 0.001$). In the investigation of the intestinal transit test, the percent inhibition of all aqueous extracts and 95 % ethanol extracts (1 g/kg bw and 0.5 g/kg bw), compound I (5 mg/kg bw) and compound II (6 mg/kg bw) on intestinal transits were higher than that of standard drug loperamide ($p < 0.001$). From these results, the anti-diarrhoeal index in percents for aqueous extracts (4g/kg bw, 2g/kg bw, 1g/kg bw), 95% ethanol extract (1g/kg bw), compound I (5mg/kg bw), compound II (6mg/kg bw) and standard drug loperamide (6mg/kg bw) were found to be 175.82 %, 168.01 %, 115.44 %, 238.53 %, 146.17 % and 285.99 % and 104.3 % respectively. It indicated that both extracts, compound I and compound II were more effective than standard drug loperamide in antidiarrhoeal activity since the higher the anti-diarrhoeal index (%), the more potent in antidiarrhoeal activity. It may be inferred that aqueous and ethanolic extracts of flower of YGG and isolated kaempferol may be used in the formulation of antidiarrhoeal medicine.

Keywords : *Gardenia coronaria* Buch-Ham., Yin-gat-gyi, anti-diarrhoeal activity

Introduction

Diarrhoea is one of the most popular disease in all over the world and it caused several millions of deaths in the world annually. In developing countries, they are the most common causes of morbidity and mortality. Some medicinal plants possess the property to cure diarrhoeal disease. The present work deals with the evaluation of anti-diarrhoeal activity of the selected medicinal plant.

Gardenia coronaria Buch-Ham., (Rubiaceae family) locally known as Yin-gat-gyi in Myanmar is a small to medium-sized but tall, deciduous tree, 7.6-9 m high. Leaves are subsessile, 15-30 cm long, obovate, shortly acuminate, shining on both surfaces. Flower are large, subsessile, terminal, white, changing to yellow, fragrant. Fruit is 2.5 cm long, ellipsoid, 5-ribbed (Uddin, 2013). Flowering period is January to April and fruiting period is February to August (Kyaw Soe and Tin Myo Ngwe, 2004). It is naturalized or cultivated throughout Myanmar for its fragrant flower (Silva and Gol, 1997).

It is used for haemoptysis, haematemesis and melena, diarrhoeal diseases, skin disorders, as antiseptic and oedema (Medicinal Plants of

Myanmar, 2010). Flower is used for pyrexia due to either biliary or chest infections or blood dyscrasia and diseases related to microorganisms. Fruit is used for cough; mucolytic (Kyaw Soe and Tin Myo Ngwe, 2004).

Materials And Methods

Sample collection

The flower and bark of *Gardenia coronaria* Buch-Ham. (Yin-gat-gyi, YGG) were collected from Chaungzone Township, Mon State. After washing with water, the collected samples were dried at room temperature. The dried samples were cut into small pieces and ground into powder by a grinding machine. These powdered samples were stored separately in air-tight containers.

Preparation of various crude extracts from YGG

The various crude extracts (PE, 95%EtOH, EtOAc and H_2O) were prepared by successive soxhlet extraction method. The extracts were concentrated by vacuum rotatory evaporator under reduced pressure to yield PE, 95% EtOH, EtOAc and H_2O extracts. The crude extracts were used to test antimicrobial and antidiarrhoeal activities and to isolate some bioactive organic constituents.

Isolation and characterization of phytochemical constituents

The EtOAc extract (5 g) of flower of YGG was separated by silica gel column chromatography with PE:EtOAc gradient elutions (9:1 to EtOAc only) to give two compounds: (I, 0.004 %, colourless needle shape and II, 0.002 %, yellow amorphous form). From the separation of EtOAc extract (5 g) of bark of YGG, one steroid compound: (III, 0.0003%, white powder form) was isolated with PE:EtOAc solvent systems (9:1 to 1:1). Then the isolated compounds were characterized by melting points, R_f values, solubilities and some chemical tests such as treating with 5% H_2SO_4, vanillin-H_2SO_4, anisaldehyde-H_2SO_4, Liebermann-Burchard reagent on TLC chromatogram followed by treating with 1% $FeCl_3$ solution.

Identification of some phytochemical constituents

The structures of isolated compounds were identified by modern spectroscopic techniques such as UV, FT IR, and ^{1}H NMR.

Screening of Antidiarrhoeal Activity

Materials

Ethanolic extract of flower of YGG, 90 albino mice of both sexes (body weight 20-30 g), mice cages, castor oil (MPF), loperamide HCl (Picco Pharma Co.Ltd), 0.9 % NaCl (normal saline, Euro-med laboratories PHIL., INC), 10 % charcoal powder, syringes and needles, intragastric dosing cannula (18 gauge) were used.

Method

Castor oil-induced diarrhea

According to the method of Awounters, 1978, mice were divided into ten groups of six animals each. Group 1 received saline (10 mL/kg bw orally) served as control group, group 2 received loperamide (6 mg/kg bw orally) served as standard group, served as group 3, 4 and 5 received aqueous extracts (4, 2, 1 g/kg bw, orally), groups 6, 7 and 8 received 95 % ethanol extracts (2, 1, 0.5 g/kg bw, orally), group 9 received compound I (5 mg/kg bw, orally) and group 10 received compound II (6 mg/kg bw, orally) respectively 1 h before castor oil administration. Diarrhoea was induced by administering 10 mL/kg body weight of castor oil orally. The onset of diarrhea, number of diarrhoeal droppings were counted hourly for 4 h, mean of the dropping passed by the treated groups were compared with that of the control group consisted of animals given an oral administration of saline (10 mL/kg bw).

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al*., 1976. Overnight fasted mice were divided into ten groups of six animals each. Group 1 received normal saline, orally (10 mL/kg bw), served as a control, group 2 received loperamide (6 mg/kg bw) and groups 3, 4 and 5 received aqueous extracts (4, 2, 1 g/kg bw, orally), groups 6, 7 and 8 received 95 % ethanol extracts (2, 1, 0.5 g/kg bw, orally), group 9 received compound I (5 mg/kg bw, orally) and group 10 received compound II (6 mg/kg bw, orally)

respectively, 1 h before the oral administration of castor oil. Thirty minutes after administration of castor oil, the mice were sacrificed and the small intestine was removed after tying both ends with thread, and weighed. The intestinal contents from each intestine were collected by milking into a beaker and their volume was measured. The intestine was reweighted and the difference between full and empty intestine was calculated as the weight of fecal matter.

Castor oil-induced small intestinal transit

According to the method of Mascolo *et al*., 1994, mice were fasted for 18 h, and divided into ten groups of six animals each. Group 1 received normal saline (10 mL/kg bw, orally), group 2 received loperamide (6 mg/kg bw, orally), groups 3, 4 and 5 received aqueous extracts (4, 2, 1 g/kg bw, orally), groups 6, 7 and 8 received 95 % ethanol extracts (2, 1, 0.5 g/kg bw, orally), group 9 received compound I (5 mg/kg bw, orally) and group 10 received compound II (6 mg/kg bw, orally) respectively, 1 hour before administration of castor oil. The maker (10 mg/kg body weight of 10 % charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The mice was sacrificed 30 min after maker administration and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum (i.e., % intestinal transit).

$$\% \text{ intestinal transit} = \frac{\text{Distance travelled by charcoal mea}}{\text{Total length of small intestine}}$$

***In vivo* antidiarrhoeal index of *Gardenia coronaria* Buch-Ham.**

The *in vivo* antidiarrhoeal index of YGG was expressed according to the following formula.

$$\text{ADI } in\ vivo = \sqrt{D_{freq} x\ G_{mag} x\ P_{freq}}$$

where, D_{freq} = delaying defecation time of diarrhoea onset, in percent of control

G_{mag} = gut meal travel distance reduction, in percent of control

P_{freq} = purging frequency as number of stool reduction, in percent of control (Aye Than *et al*., 1989)

Results and Discussion

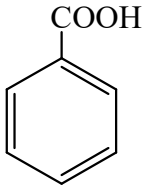
Preparation of Crude Extracts

The various crude extracts by using successive solvent extraction method were prepared as PE (3.21 %), EtOH (25.07 %), EtOAc (10.08 %) and H_2O (11.89 %) in flower as well as PE (0.29 %), EtOH (11.13 %), EtOAc (3.48 %) and H_2O (4.68 %) in bark.

Identification of Isolated compounds

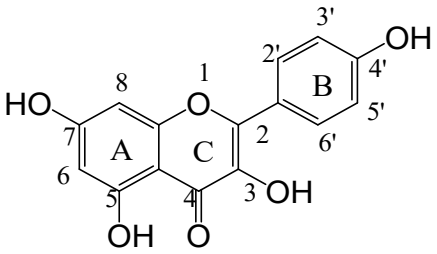
Compound I from flower of YGG

Benzoic acid (7.5 mg, 0.004 %), ^{1}H NMR (400 MHz, $CDCl_3$), δ = 8.12 (d, J = 7.2 Hz), 7.62 (t, J = 7.2 Hz), 7.48 (t, J = 7.6 Hz)



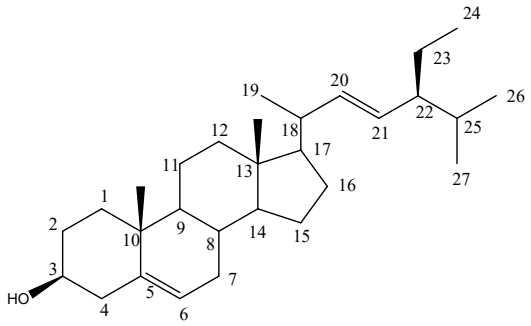
Compound II from flower of YGG

Kaempferol (4.6 mg, 0.002 %, 275-280°C), UV: (MeOH), λ_{max} = 269,322, 367 nm (MeOH/NaOMe), λ_{max} = 277,320,409 nm, (MeOH/$AlCl_3$), λ_{max} = 269,340,424 nm, (MeOH/$AlCl_3$/HCl), λ_{max} = 265,304,359,425 nm, (MeOH/NaOAc), λ_{max} = 269,305,370 nm, (MeOH/NaOAc/H_3BO_3), λ_{max} = 266,324,364 nm, FT IR : (KBr), ν_{max} = 3450 (broad) (ν_{OH}), 3017($\nu_{=CH}$), 1658 ($\nu_{C=O}$), 1612, 1507 ($\nu_{C=C}$), 1458 (δ_{CH}), 1381(δ_{OH}), 1255(ν_{C-O}), 1174 (ν_{C-O}), 885 cm^{-1}(δ_{CH}), ^{1}H NMR: (400MHz, $CDCl_3$), δ = δ_H 6.18 (1H, d, J = 2.0 Hz), δ_H 6.39 (1H, d, J = 2.0 Hz), δ_H 8.06 (1H, d, J = 8.9 Hz), δ_H 6.89 (1H, d, J = 8.9 Hz), δ_H 6.89 (1H, d, J = 8.9 Hz), δ_H 8.06 (1H, d, J = 8.9 Hz)



Compound III from bark of YGG

Stigmasterol (1.781 mg, 0.0003%, 167-170°C), FT IR: (KBr), ν_{max} = 3450-3050 (broad) (ν_{OH}), 2935 (ν_{CH}), 1650 ($\nu_{C=C}$), 1463 (δ_{CH}), 1377 (δ_{symCH}), 1049 (ν_{C-O}), 958 [δoop(=CH)], 839-800 cm^{-1}[δoop(=CH)]



Antidiarrhoeal activity

Antidiarrhoeal activity of the isolated compounds, 95 % ethanol and watery extracts of flower of YGG were screened by using castor oil-induced mice models.

Castor oil-induced diarrhoea

In the investigation of castor oil-induced diarrhoea, it was found that the mean frequency of aqueous extract with 4 g/ kg bw dose (6.33 ± 0.80) is the lowest in all of the aqueous extracts (4 g/kg bw, 2 g/kg bw, 1 g/kg bw). The percent inhibition of defecation of aqueous extracts (1 g, 2 g, 4 g/kg bw) of YGG were 40.81 %, 46.37 %, 69.61 %, respectively (Table 1). When the dose is increased, the mean frequency of diarrhoea become reduced. These results showed reduction in dose dependent manner.

Similarly, it was found that the mean frequency of 95 % ethanol extract with 1 g/ kg bw dose (5.33 ± 0.85) is the lowest in all of the 95 % ethanol extracts (0.5 g/kg bw, 1 g/kg bw, 2 g/kg bw). The percent inhibition of defecation of aqueous extracts (0.5 g, 1 g, 2 g/kg bw) of YGG were 32.79 %, 74.41 %, 35.19 %, respectively (Table 1). Although there were more increase in dose, no reduction in frequencies of diarrhoea. These results showed reduction in dose independent manner. Therefore, it was deduced that the medium dose (1 g/kg bw) of 95 % ethanol extract was found to possess the best activity in all of the ethanol extracts.

The percent inhibitions of defecation of compound I (5 mg/kg bw) and compound II (6 mg/kg bw) of YGG were found to be 57.61 % and 84.78 %, respectively (Table 1). The mean frequency of defecation in 4 hour and percent inhibition are shown in Table 1 and Figure 1. Based on the results of all samples, it was also found that the mean frequency of diarrhoea increased with increase in time after drug adniaintration.

Table 1. Effect of Extracts and Isolated Compounds from Flower of YGG on Castor Oil-Induced Diarrhoea in Mice

Treatment	Mean defecation in 4 h	% Inhibition of defecation
Control	20.83 ± 1.51	
Loperamide 6 mg/kg	6.33± 2.59*	69.61
Aqueous extract 4 g/kg	6.33 ± 0.80	69.61
Aqueous extract 2 g/kg	11.17 ± 2.39*	46.37
Aqueous extract 1 g/kg	12.33 ± 1.31**	40.81
Ethanolic extract 2 g/kg	13.5 ± 0.76*	35.19
Ethanolic extract 1 g/kg	5.33 ± 0.84*	74.41
Ethanolic extract 0.5 g/kg	14.00 ± 0.58*	32.79
Compound I 5 mg/kg	8.83 ± 1.30	57.61
Compound II 6 mg/kg	3.17 ± 0.65*	84.78

*$P < 0.001$, **$P < 0.005$

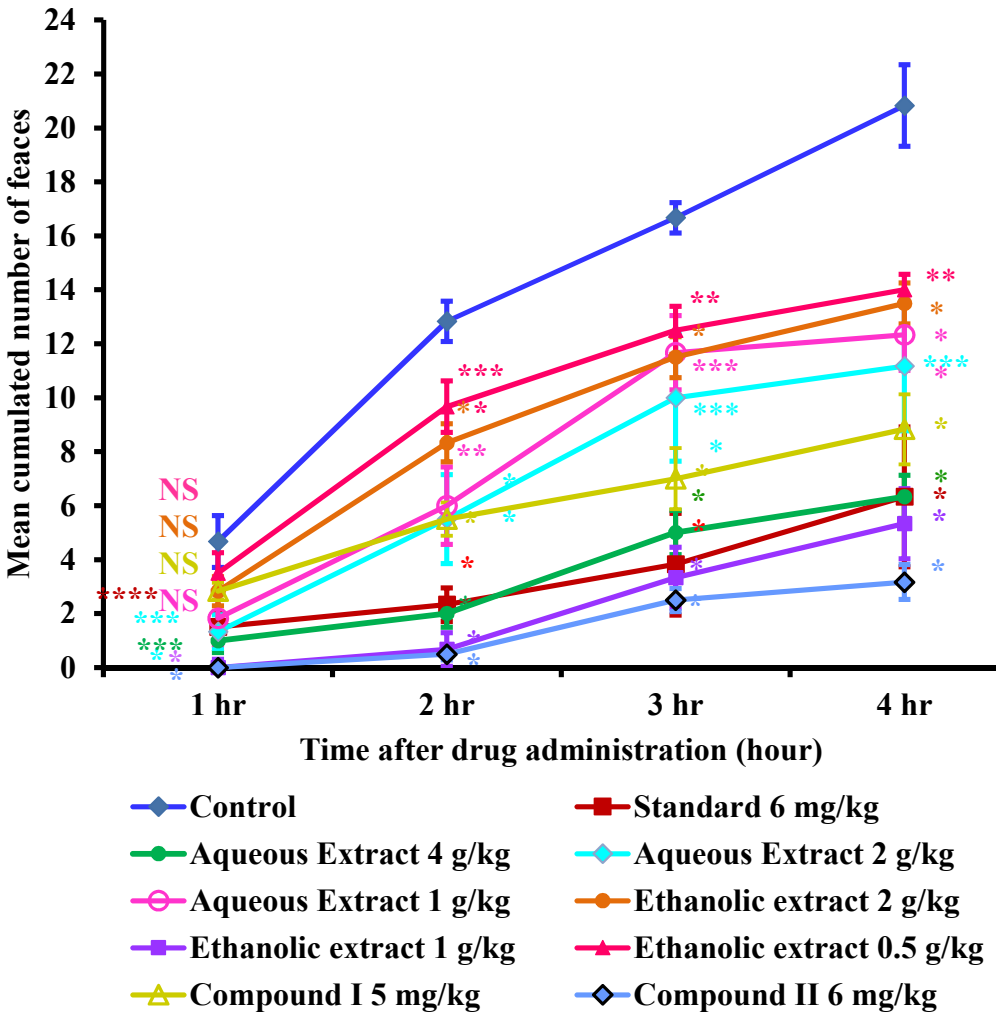


Figure 1. Comparative effects of aqueous extract of (4 g/kg bw, 2 g/kg bw, 1g/kg bw), 95 % ethanolic extract of (2 g/kg bw, 1g/kg bw, 0.5 g/kg bw), compound I, compound II of flower of YGG and standard drug loperamide on mice with castor oil-induced diarrhea at various time intervals $^{*}P<0.001$, $^{**}P<0.005$, $^{***}P<0.01$, $^{****}P<0.05$

Castor oil-induced enteropooling

In the study of castor oil-induced enteropooling, it was investigated that the mean weight (0.87 ± 0.11 g) and volume (0.37 ± 0.06 mL) of intestinal content in aqueous extract 4 g/kg bw treated group were reduced more than that of the remaining aqueous extracts (2 g/kg bw and 1 g/kg bw) (Table 2). There was dose dependent reduction in fecal weight and volume. It was found that the more increase in doses, the more reduction in the fecal weight and volume. So, the extract has negative relationship between dose and effect (Figures 2 and 3).

Similarly, it was investigated that the mean weight (0.79 ± 0.25g) and volume (0.40 ± 0.06 mL) of intestinal content in 95 % ethanol extract with 1 g/kg bw dose were reduced more than that of the remaining 95 % ethanol extracts (0.5 g/kg bw and 2 g/kg bw). There was dose independent reduction in fecal weight and volume. The more increase in doses, the less reduction in the fecal weight and volume (Figures 2 and 3).

The mean weight (g) and volume (mL) of intestinal content for compound I treated group were 0.87 ± 0.11 g ($p < 0.05$) and 0.32 ± 0.07 mL ($p< 0.01$) respectively and that of compound II treated group were 0.33 ± 0.06 g ($p < 0.001$) and 0.17 ± 0.04 mL ($p< 0.001$) respectively and the results were significantly when compared with the control group.

Comparative weight (g) and volume (mL) of intestinal content of the control, loperamide, compound I, compound II and different doses of aqueous and ethanol extracts of YGG on castor oil-induced enteropooling in mice are shown in Table 2 and Figures 2 and 3.

Table 2. Effect of Compound I, Compound II, 95% Ethanol and Watery Extract of flower of YGG on Castor Oil-induced Enteropooling in Individual Mice

Treatment	Weight of intestinal content (g)	Volume intestinal content (mL)
Control	1.43 ± 0.09	0.82 ± 0.07
Loperamide 6 mg/kg	0.39 ± 0.08*	0.18 ± 0.03*
Aqueous extract 4 g/kg	0.87 ± 0.11**	0.37 ± 0.06*
Aqueous extract 2 g/kg	1.06 ± 0.09***	0.53 ± 0.09***
Aqueous extract 1 g/kg	1.15 ± 0.09****	0.55 ± 0.05***
Ethanolic extract 2 g/kg	0.90 ± 0.10**	0.40 ± 0.07**
Ethanolic extract 1 g/kg	0.79 ± 0.25****	0.40 ± 0.06*
Ethanolic extract 0.5 g/kg	1.02 ± 0.07**	0.52 ± 0.06****
Compound I 5 mg/kg	0.87 ± 0.11****	0.32 ± 0.07***
Compound II 6 mg/kg	0.33 ± 0.06*	0.17 ± 0.04*

*P < 0.001, **P < 0.005, ***P < 0.01, ****P < 0.05

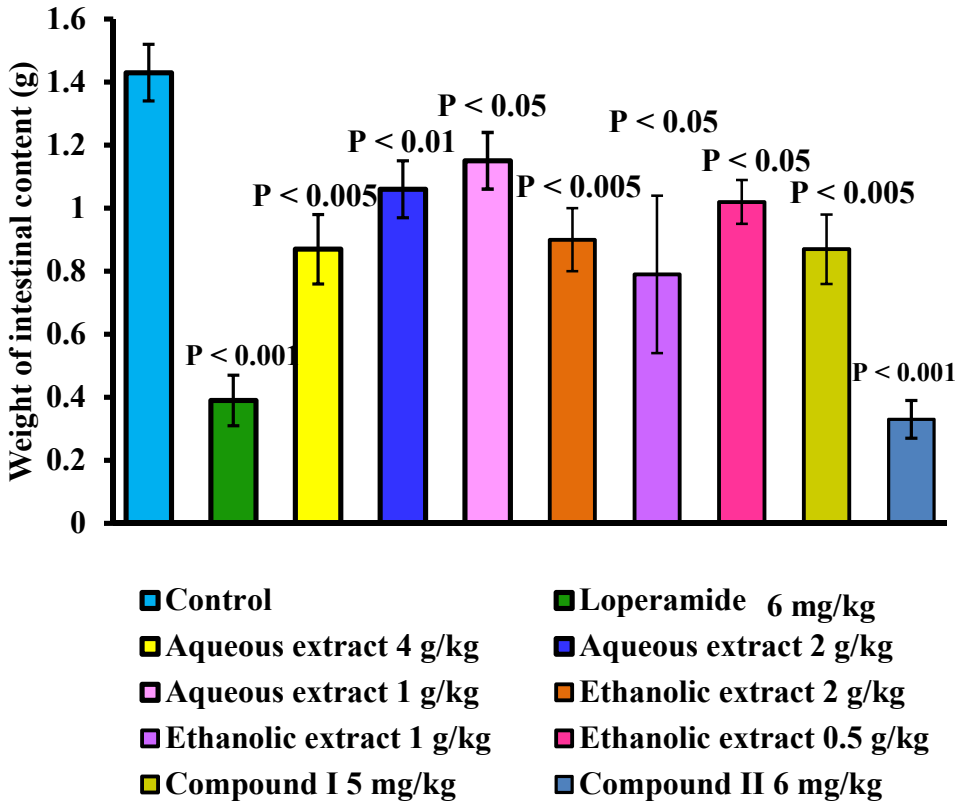


Figure 2. Comparative antidiarrhoeal effects (weight of intestinal content) of flower of YGG aqueous extract (4 g/kg bw, 2 g/kg bw, 1g/kg bw), 95 % ethanolic extract (2 g/kg bw, 1 g/kg bw, 0.5 g/kg bw), compound I (5 mg/kg bw), compound II (6 mg/kg bw) and standard drug loperamide (6 mg/kg bw) on castor oil-induced enteropooling in mice

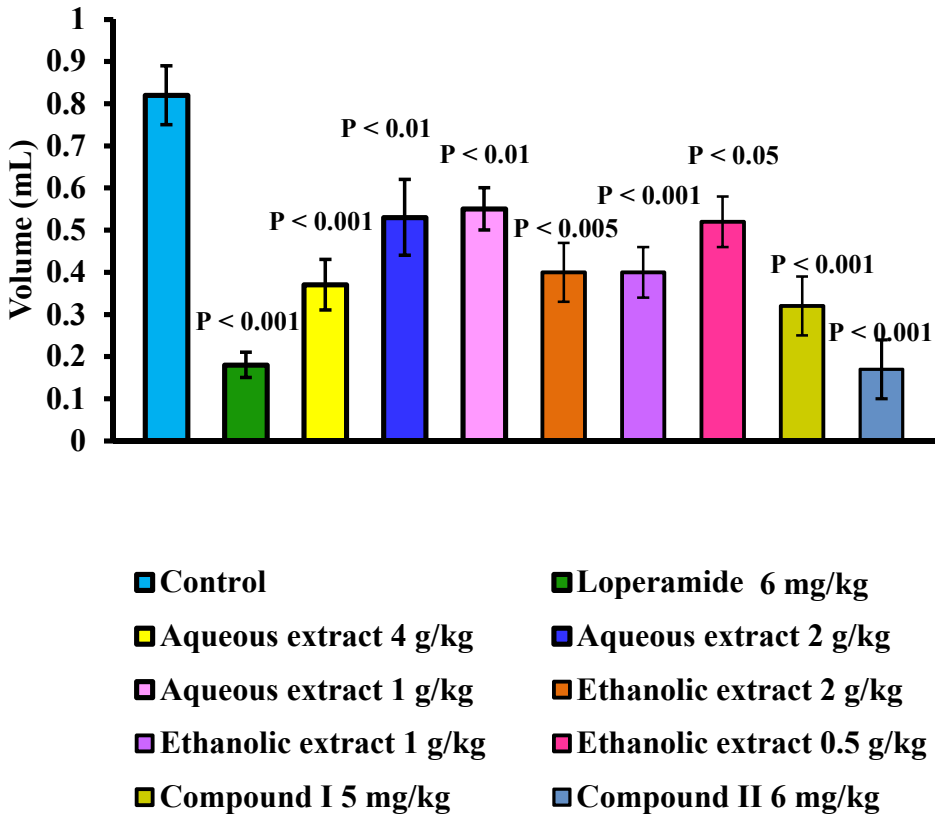


Figure 3. Comparative antidiarrhoeal effects (volume of intestinal content) of flower of YGG aqueous extract (4 g/kg bw, 2 g/kg bw, 1g/kg bw), 95 % ethanolic extract (2 g/kg bw, 1 g/kg bw, 0.5 g/kg bw), compound I (5 mg/ kg bw), compound II (6 mg/kg bw) and standard drug loperamide (6 mg/kg bw) on castor oil-induced enteropooling in mice

From the table and figure, it can be seen that the mean weight (0.33± 0.06 g) and volume (0.17± 0.04 mL) of intestinal content of compound II with 6 mg/kg bw dose the lowest in all of the samples tested. In addition, it was found to be more potent than that of the standard loperamide.

Castor oil-induced small intestinal transit

In the small intestinal transit test, it was found that the intestinal transit (25.29 ± 2.97 % ($p < 0.001$) of charcoal and inhibition of intestinal transit (74.71 ± 2.97 % ($p < 0.001$) of individual mice in aqueous extract 4 g/kg bw treated group) were better than that of the remaining samples (Table 3). The more increase in dose, the more reduction in percent intestinal transit. The results showed reduction in dose dependent manner (Figures 4 and 5).

It was also found that the intestinal transit 38.48 ± 5.29 % ($p < 0.001$) of charcoal and inhibition of intestinal transit 61.53 ± 5.29 % ($p < 0.001$) of individual mice in 95 % ethanol extract with 1 g/kg bw dose were better than that of the remaining samples. Although there was more increase in dose, no reduction in percent intestinal transit. The results showed reduction in dose independent manner (Figures 4 and 5).

Comparative intestinal transit (%) and inhibition of intestinal transit (%) of the control, loperamide, compound I, compound II, different doses of aqueous and ethanol extracts of YGG on castor oil-induced intestinal transit in mice are shown in Table 3, Figures 4 and 5. In the present work, the percent intestinal transit of compound I (5 mg/kg bw) was reduced more than the those of remaining samples. Therefore, the (%) inhibition of intestinal transit of compound I was the highest in all of the samples tested. According to castor oil-induced small intestinal transit test, it was found that the more decrease in intestinal transit (%) and the more increase in transit inhibition (%), the better is its antidiarrhoeal activity.

Table 3. Effect of Compound I, Compound II, 95 % Ethanol and Watery Extracts of flower of YGG On Castor Oil-induced Intestinal Transit in Individual Mice

Treatment	% Intestinal transit	% Inhibition
Control	71.77 ± 2.04	28.23 ±2.04
Loperamide 6 mg/kg	46.99 ± 5.31*	53.01 ±5.31*
Aqueous extract 4 g/kg	25.29 ± 2.97*	74.71 ± 2.97*
Aqueous extract 2 g/kg	36.07 ± 4.35*	63.93 ± 4.35*
Aqueous extract 1 g/kg	35.39 ± 3.21*	64.61 ± 3.21*
Ethanolic extract 2 g/kg	47.75 ±4.37*	52.25 ±4.37*
Ethanolic extract 1 g/kg	38.48 ±5.29*	61.53 ±5.29*
Ethanolic extract 0.5 g/kg	42.55 ±1.62*	57.45 ±1.62*
Compound I 5 mg/kg	16.47 ± 1.92*	83.53 ± 1.92*
Compound II 6 mg/kg	22.96 ± 4.89*	77.04 ± 4.89*

*$P < 0.001$

From Figures 4 and 5, it can be seen that the mean intestinal transit (16.47 ± 1.92 % ($p < 0.001$)) and the mean of transit inhibition (83.53 ± 1.92 % ($p < 0.001$)) of compound I were the best in all of the samples tested.

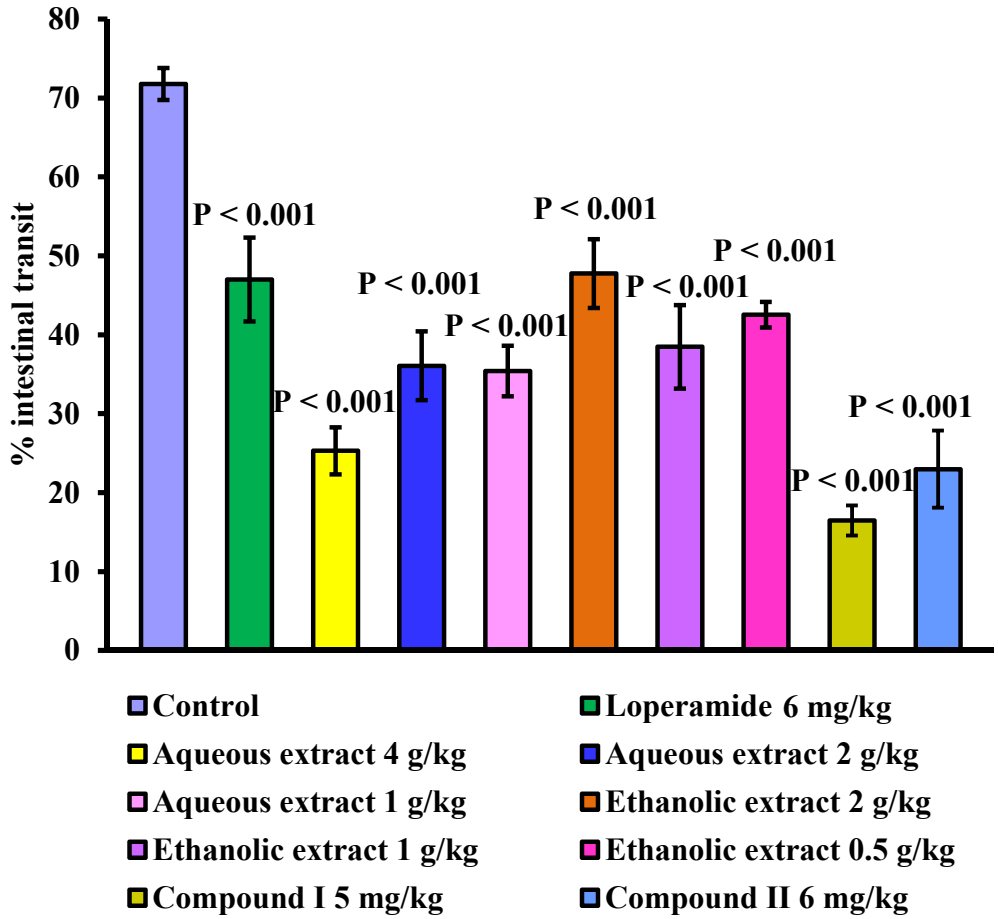


Figure 4. Percentage intestinal transit of aqueous extract (4 g/kg bw, 2 g/kg bw, 1g/kg bw), 95 % ethanolic extract (2 g/kg bw, 1 g/kg bw, 0.5 g/kg bw), compound I (5 mg/kg bw), compound II (6 mg/kg bw) of flower of YGG and standard drug loperamide (6 mg/kg bw) on castor oil-induced intestinal transit in mice

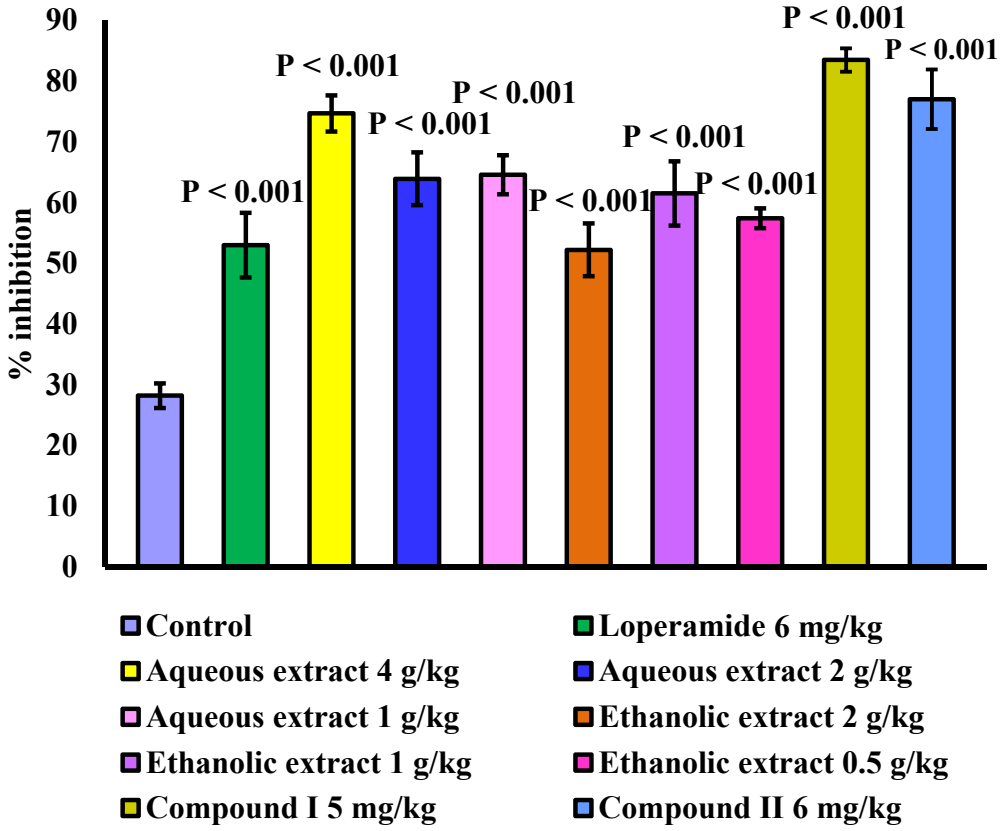


Figure 5. Percentage inhibition effect of aqueous extract (4 g/kg bw, 2 g/kg bw, 1g/kg bw), 95 % ethanolic extract (2 g/kg bw, 1 g/kg bw, 0.5 g/kg bw), compound I (5 mg/kg bw), compound II (6 mg/kg bw) of flower of YGG and standard drug loperamide (6 mg/kg bw) on castor oil-induced inhibition in mice

Table 4. Antidiarrhoeal efficacies of *Gardenia coronaria* Buch-Ham. on *in vivo* mouse models

Treatment	Delaying defaecation time of onset in mice, min	Gut meal travel distance in mice, cm	Purging frequency in mice, in number of stool	*In vivo* antidiarrhoeal index (%)
Control	3.50 ± 0.85	42.05 ± 1.39	20.83 ± 1.51	0
Loperamide 6 mg/kg	19.67 ± 10.75	27.33 ± 2.89*	6.33 ± 2.59*	104.30
Aqueous extract 4 g/kg	49.50 ± 25.34	17.07 ± 2.04****	6.33 ± 0.80	175.82
Aqueous extract 2 g/kg	68.17 ± 35.82	18.78 ± 2.51*	11.17 ± 2.39***	168.01
Aqueous extract 1 g/kg	30.17 ± 13.44	21.25 ± 2.20*	12.33 ± 1.31**	115.44
Ethanolic extract 2 g/kg	13.33 ± 7.23	27.88 ± 2.49*	13.5 ± 0.76**	69.32
Ethanolic extract 1 g/kg	130 ± 24.81*	20.83 ± 2.72*	5.33 ± 0.84*	238.53
Ethanolic extract 0.5 g/kg	19.5 ± 12.47	23.98 ± 1.59*	14 ± 0.58**	86.36
Compound I 5 mg/kg	29.5 ± 8.36***	11.37 ± 1.27*	8.83 ± 1.3*	146.17
Compound II 6 mg/kg	139.17 ± 14.27*	12.12 ± 2.51*	3.17 ± 0.65*	285.99

*$P < 0.001$, **$P < 0.005$, ***$P < 0.01$, ****$P < 0.05$

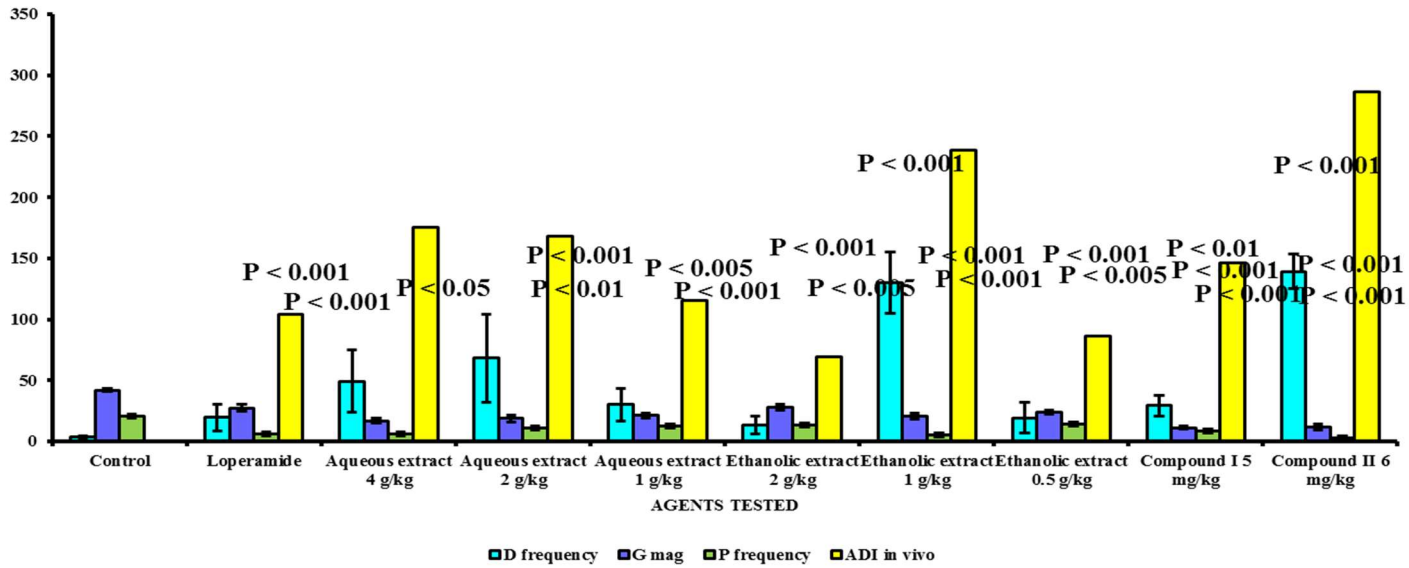


Figure 6. Comparative anti-diarrhoeal efficacies of flower of YGG aqueous extract (4 g/kg bw, 2 g/kg bw, 1g/kg bw), 95 % ethanolic extract (2 g/kg bw, 1 g/kg bw, 0.5 g/kg bw), Compound I (5 mg/kg bw), Compound II (6 mg/kg bw) and standard drug loperamide (6 mg/kg bw) on *in vivo* mouse model

D frequency = Delaying defecation time of onset in mice, in min

G mag = Gut meal travel distance in mice, in cm

P frequency = Purging frequency in mice, in number of stool

ADI *in vivo* = *In vivo* antidiarrhoeal index, in percent

Antidiarrhoeal index (ADI) was calculated by the method of Aye Than *et al*., 1989. ADI of aqueous extract (1, 2 and 4 g/kg bw) were 115.44 %, 168.01 % and 175.82 %, respectively (Table 4 and Figure 6). This means that when the dose was increased, the antidiarrhoeal index was also increased. However, ADI of 95 % ethanol extract (0.5, 1 and 2 g/kg bw) were 86.36 %, 238.53 % and 69.32 %, respectively (Table 4 and Figure 6). This means that when the dose was increased, the antidiarrhoeal index was decreased. Nevertheless, it was found that the higher the index, the better is the antidiarrhoeal activity. Therefore, it could be deduced that the antidiarrhoeal activity of compound II (6 mg/kg bw) with 285.99 % ADI value was the best in all of the samples tested and it was found to be considerably more potent than the standard loperamide (104.3 % ADI value).

The results of the present study showed that the flower of YGG produced significant frequency, enteropooling and intestinal transit. This also indicated showed that YGG has antisecretory and antimotility effects. So, *in vivo* test of this study revealed that the antidiarrhoeal activity of 6 mg/kg bw of compound II from flower of YGG was comparable with that of loperamide 6 mg/kg on counting number of stool, enteropooling and intestinal transit.

The flower of YGG is abundant in Mon State, Myanmar. The remarkable antidiarrhoeal effect of YGG against castor oil-induced diarrhoea showed its utility in a wide range of diarrhoea.

Conclusion

Therefore, it could be deduced that YGG flower may be used for the treatment of the diseases caused by some microorganisms. In addition, it may be effective for the diseases infected by bacteria causing diarrhoea such as haemoptysis, haematemesis, melena and diarrhoeal diseases Compound I (Benzoic acid), Compound II (Kaempferol) and Compound III (Stigmasterol) were extracted from the bark of YGG. It may be inferred that aqueous and ethanolic extracts of flower of YGG and isolated kaempferol may be used in the formulation of antidiarrhoeal medicine.

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