Antidiarrhoeal, Anti-inflammatory and analgesic activities of *Symplocos racemesa* roxb. Bark

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Abstract: The antidiarrheal activity of the drug *Symplocos racemosa* was performed *in-vivo* on isolated rabbit intestine. The effects of crude extract and fractions were observed at different doses. The overall response of the crude extract on isolated tissue of rabbit intestine was decreased in the tone of smooth muscle. Further studies were carried out on different fractions (ethylacetate, chloroform, *n*-butanol and aqueous) of crude extract of *S. racemosa*. The standard drugs were also used for further screening of the fractions of *S. racemosa*. Hot plate, writhing test, formalin test and carrageenan-induced paw edema in mice and rats were performed for determination of analgesic and anti-inflammatory activities respectively on *S. racemosa* bark extract. The results exhibited significant anti-inflammatory and analgesic effect at 300 and 500mg/kg doses.

Keywords: *Symplocos racemosa*, antidiarrhoeal, anti-inflammatory, analgesic.

INTRODUCTION

Symplocos racemosa (Symplocaceae) is locally known as Lodh. The pinkish bark contains alkaloids loturine, colloturine and loturidine. Ash contains carbonate of soda. Large quantity of /red colouring matter is present (Ishida *et al*., 2002) while tannins are absent. The bark is acrid; cooling, digestible, astringent to the bowels and alexiteric. It is useful in eye disease, the paste of Lodh bark is applied around the eyes. Bark finely powdered and with some vehicle is dropped in to ears to stop abnormal discharge, for spongy gums and bleeding. It cures biliousness, diseases of the blood, dysentery, inflammations, vaginal discharges, leprosy and is useful in abortion and miscarriages. It is good for ulcers in the vagina (Nadkarni. 2002).

Locoracemosides A, B and C were isolated from the bark of of *Symplocos racemosa* Roxb and found active against α-chymotrypsin (Rashid *et al*., 2008). Since traditionally *S. racemosa* is very effective therefore, antidiarrhoeal, anti-inflammatory and analgesic activities were carried out on bark extract and fractions.

MATERIALS AND METHODS

Materials and methods

The 2 kg dry Bark of *S. racemosa* was collected from Karachi, Pakistan, identified and deposited in the herbarium of Department of Pharmacognosy (voucher specimen no. 20031). It was soak in sufficient amount of ethanol for 15 days. The extract was filtered and evaporated as described by Ahmad *et al.,* (2008). 30gm of crude extract was dissolved in equal volume of distilled water and ethyl-acetate to yield 3.5gm of ethyl-acetate fraction, followed by 1.2 gm chloroform, 1.6 gm n-butanol and 1.9gm aqueous fraction (Mehjabeen *et al*., 2004 and Ahmad *et al.,* 2012). All the chemicals used during experiments were purchased from Merck, Germany.

Male rabbits (1.0 to 1.kg) were used during experiments. The animals were purchased from bred stock available locally. The animals were kept in separate cages, food and water was provided as per fixed rules.

Mice (albino) and rats (Wistar) were purchased from the Animal House of Dow University of Health Sciences, Karachi and used for analgesic and anti-inflammatory activities Food and water was provided *ad libitum*.

Antidiarrhoeal activity

Antidiarrhoeal activity was carried out on isolated rabbit intestine by using a modified method of Blattner (1978). A blow on the back neck sacrificed the rabbit. The abdomen was made open immediately and caecum was pulled forward to display the length of small intestine. The intestine was then cut from animal and placed in a Petri-dish or beaker containing Tyrode's solution

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(Mehjabeen *et al*., 2004). The segments of small intestine (jejunum or ileum) about 3-4cm long were dissected immediately from isolated intestine, later it was placed in Petri-dish or beaker containing Tyrode's solution. For experimentation a piece of isolated smooth muscles was mounted in an organ bath of 70ml capacity, filled with Tyrode's solution. Organ bath circulating water temperature was maintained to 37°C throughout the experiment. The perfusion solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The intestine segment was allowed to equilibrate before starting the experiments. The spontaneous movements of intestine were recorded on Oscillograph or polygraph using isotonic transducer (Blattner *et al*., 1978). To determine the effects of plant extract on spontaneous movements of intestine, crude extract was dissolved distilled water and thereafter, it was added to the organ bath after equilibration period (Ahmad *et al*., 2012). The effects of crude extracts on the contraction and relaxation pattern of isolated rabbit intestine (smooth muscles) are recorded in tables. The doses of crude extract were 1, 5, 10, 15, 20 and 25mg/ml whereas the response of its ethyl-acetate, chloroform, *n*-butanol and aqueous fractions were observed at 15mg/ml.

Analgesic and anti-inflammatory activity

Mice (albino) and rats (Wistar) were used for analgesic and anti-inflammatory activities. They were kept under standard animal laboratory conditions. The Crude extract of *Symplocos racemosa* was used in 200, 300 and 500 mg/kg doses orally. For analgesic test hot plate method (Lanhers MC *et al*., 1991) and writhing test were performed (Koster *et al*., 1959). While formalin test (Tjolsen *et al*., 1992) on mice and carrageenan induced paw edema test on rats were used for the assessment of anti-inflammatory response (Winter *et al*., 1962). Acetic acid (0.6% in 0.9% saline) 0.1ml/10g mice was administered I/P after 30 minutes of test drug administration in writhing test. 20µl of 1% Formalin was introduced in right hind paw of mice in formalin induced inflammatory test. 10 ml/kg of 1% Carrageenan solution prepared in 0.9% saline solution was used to induce inflammation in rats. In hot plate test mice were placed on hot plate at 51±2ºC. The reaction time was noted in term of flicking tail or paws. The results of analgesic and anti-inflammatory activities were compared with standard drug, Aspirin, 300mg/kg.

STATISTICAL ANALYSIS

All Statistical comparisons were carried out by Student's *t-test* and results were expressed as mean ± S.E.M at $p \leq 0.05$.

RESULTS

The antidiarrhoeal activity was carried out by *in vitro* experiment using isolated rabbit intestine. The results of crude extract and fractions of *S. racemosa* were presented in table 1-2 and fig. 1-2.

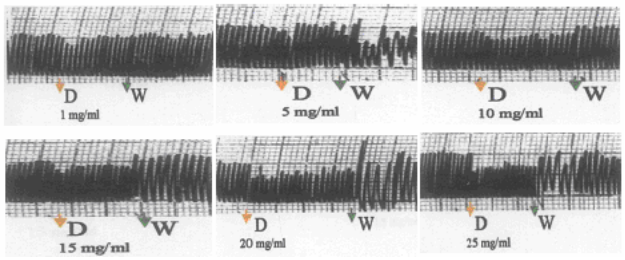


Fig. 1: Tracing of *in vitro* intestinal activity of crude extract of *S. racemosa*

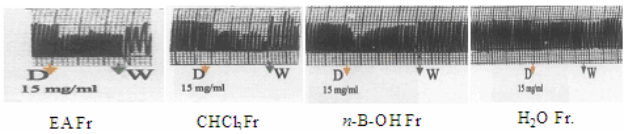


Fig. 2: Tracing of *in vitro* intestinal activity of ethylacetate, chloroform, *n*-butanol and aqueous fractions of *S. racemosa*

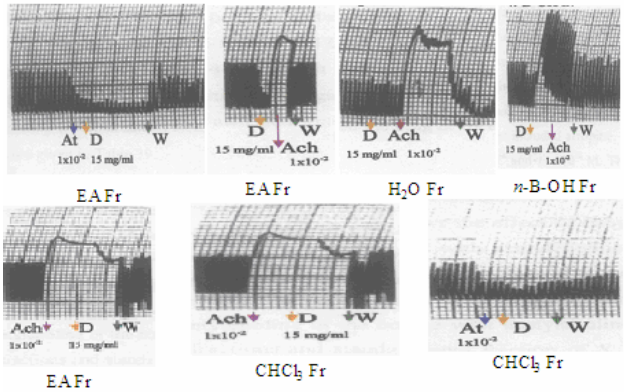
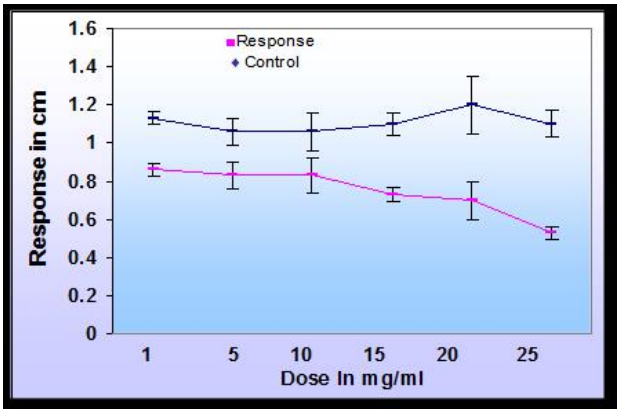


Fig. 3: Tracing of *in vitro* intestinal activity of ethylacetate, chloroform, *n*-butanol and aqueous fractions of *S. racemosa* in comparison with standard drugs (EA: ethylacetate, *n*-B-OH: *n*-butanol, H_2O: aqueous, D: dose of drug. W: washing, Fr: Fraction, At: atropine $1x10^{-2}$ M, Ach: acetylcholine $1x10^{-2}$ M).

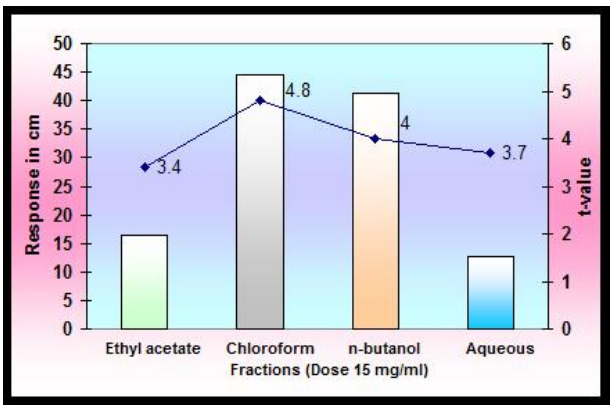
It was observed that at the dose of 1 mg/ml there was a slight decrease in the rhythmic response of intestine. This showed a negligible smooth muscle relaxing activity. At the dose of 5 mg/ml, increase in the muscle activity was found that is contraction of smooth muscle. Relaxing response of isolated tissue was observed at the dose of 10 mg/ml. The dose of 15mg/ml showed more or less same relaxing response as 10mg/ml while the relaxation of the tissue of rabbit intestine was prominent at the doses of 20 and 25 mg/ml.

The ethyl-acetate and chloroform fractions of the drug showed relaxation of isolated tissue of rabbit intestine

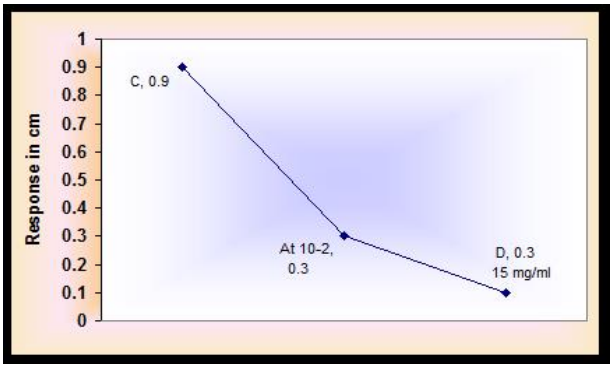
similar to the effect of crude extract 25mg/ml (table 2, fig. 2). The fractions of *S. racemosa* were also treated with standard drugs atropine and acetylcholine (fig. 3, Graph 3-7).



Graph 1: The *in vitro* antidiarrhoeal activity along with SEM of crude extract of *S. racemosa* on isolated intestine of rabbit**.**

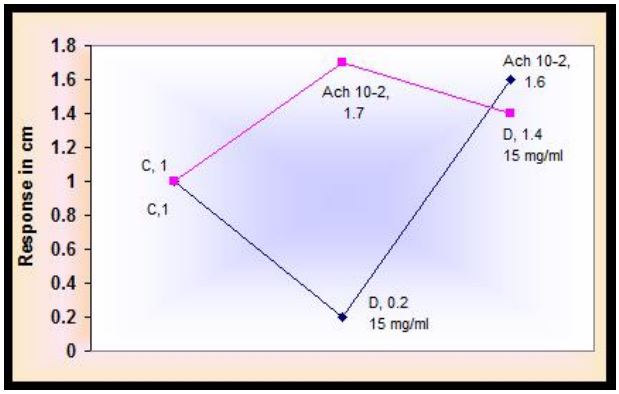


Graph 2: The *in vitro* antidiarrhoeal activity of fractions of *S. racemosa* on isolated intestine of rabbit.

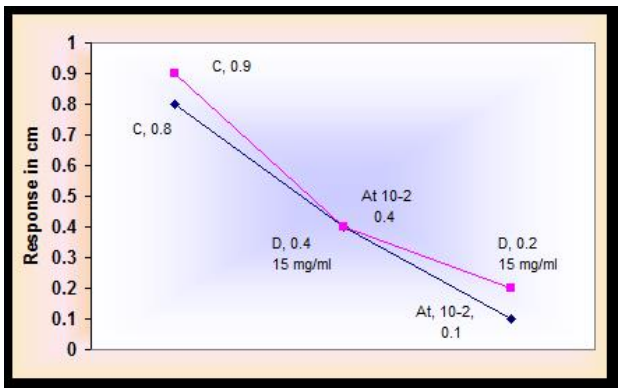


Graph 3: Effect of Ethyl-acetate fraction of *S. racemosa*, pretreated tissue with Atropine in 1 x 10^{-2} M concentration.

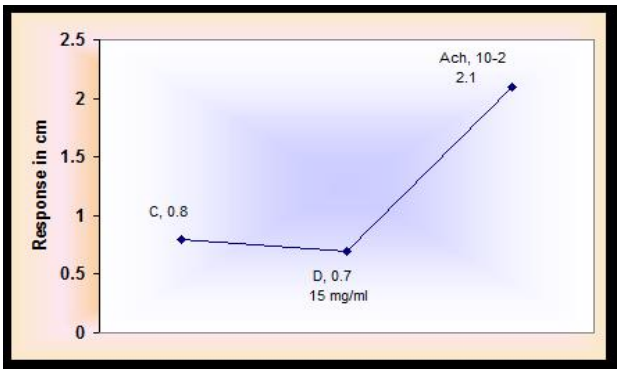
The results of analgesic activity are presented in table 3-4. *S. racemosa* produced maximum percentage of inhibition in writhing test (55.7 and 59.01 at 1st and 2nd phase respectively). Aspirin was used as reference standard drug (table 3). The maximum analgesic response with hot plate activity was observed at 2.5 hrs. (table 4). *S. racemosa* also exhibited its anti-inflammatory response at 500 mg/kg which was more significant than aspirin in the second phase of formalin induced licking and biting (table 5). The results of carrageenan induced inflammation test also exhibited anti-inflammatory effect. The maximum percentage of inhibition of paw edema was observed with 500mg/kg at 2 hrs (table 6).



Graph 4: Effect of Ethyl-acetate fraction of *S. racemosa*, pretreated and post treated tissue with Acetylcholine in 1 x 10^{-2} M concentration.



Graph 5: Effect of chloroform fraction of *S. racemosa*, pre and post-treated tissue with Atropine 1 x 10^{-2} M concentration.



Graph 6: Effect of Aqueous fraction of *S. racemosa*, post-treated tissue with Acetylcholine in 1 x 10^{-2} M concentration.

Table 1: Dose related antidiarrhoeal response of crude extract of *S. racemosa* on isolated rabbit intestine.

Dose (mg/ml)	Control (cm)	Response (cm)	Response in Percentage	t- value
01	1.13±0.034	0.86±0.034	23.89	5.4**
05	1.06±0.07	0.83±0.07	21.69	2.23*
10	1.06±0.121	0.83±0.09	21.69	1.5*
15	1.1±0.058	0.73±0.034	33.64	5.4**
20	1.2±0.153	0.7±0.1	41.7	2.73*
25	1.1±0.07	0.53±0.034	51.8	7.3**

The results are expressed in ± S.E.M, at P≤0.05, * Significant, **moderate significant, ***highly significant

Table 2: Effects of different fractions of *S. racemosa* on isolated rabbit intestine.

Fractions	Dose (mg/ml)	Control (cm)	Response (cm)	Response in Percentage	t- value
Ethylacetate	15	1.03±0.034	0.86±0.034	16.5	3.4*
Chloroform	15	1.03±0.034	0.57±0.09	44.6	4.8*
n- butanol	15	0.97±0.034	0.57±0.09	41.23	4*
Aqueous	15	1.03±0.034	0.9±0	12.62	3.7*

Table 3: Analgesic activity (Acetic acid induced writhing) of crude extract of *S. racemosa*

Treatment	Dose mg/kg orally	Mean No. of Writhes ± S.E.M		Inhibition (%)	
		1st phase	2nd phase	1st phase	2nd phase
Control	0.5 ml Saline	79±3.67	61±3.11	-	-
S. racemosa	200	61±2.31	47±2.26	22.78	22.9
	300	55±1.394	33±0.23	30.4	45.9*
	500	35±1.34	25 ± 0.99	55.7**	59.01**
Aspirin	300	28±1.29	21±1.03	64.6**	65.5**

Table 4: Hot plate activity of crude extract of *S. racemosa*

Treatment	Dose mg/kg orally	Mean No. of Licking and biting ±S.E.M		Inhibition (%)	
		1st phase	2nd phase	1st phase	2nd phase
Control	0.5 ml saline	75±2.55	55±1.68	-	-
Crude extract *S. racemosa*	200	62±2.18	40±2.01	17.33	27.27
	300	57±2.08	35±1.91	24	36.36*
	500	51±1.29	27±1.05	32	50.90**
Aspirin	300	44±2.11	31±1.99	41.3*	43.63**

Table 5: Anti-inflammatory activity (formalin induced inflammation) of Crude extract of *S. racemosa*

Variation flicking time with ± SEM (Time in sec at 55±1ºC)										
Dose mg/kg	0hr	0.5hr	1hr	1.5hrs	2hrs	2.5hrs	3hrs	3.5hrs	4hrs	4.5hrs
Control	9.6± 2.11	10 ± 2.02	10.2 ±1.98	11.1± 1.81	10.6 ± 1.85	10.1± 1.96	10.1 ±2.10	9.8± 1.66	10.2± 1.55	9.8± 1.55
S. racemosa 200mg/kg	11.2 ±1.09	12.2 ± 1.08	12.4 ±1.76	13.1± 1.55	13.4± 1.06	12.7± 1.75	12.1 ±1.56	10.2 ±1.07	10.2 ±1.66	10.1 ±1.66
S. racemosa (300mg/kg)	10.1 ±1.43	13 ± 1.65	12 ± 1.85	13.5± 2.06	13.1± 2.04	13.7*± 1.34	12.9 ±1.44	12.5 ±1.85	11.2 ±1.05	11.3 ±1.55
S. racemosa 500mg/kg	9.5 ± 1.22	12.5 ± 2.11	13.1 ±2.82	14.2± 1.86	14.5± 2.01	15.2*± 2.05	14.2 ±1.87	13.5 ±1.45	12.8 ±2.75	12.6 ±2.44
Aspirin 300mg/kg	10.6 ±1.01	15 ± 2.22	16 ± 1.32	16.4± 1.65	18.5** ± 1.56	17.5*± 1.69	18 ± 2.11	18.5 ± 2.32	18.6**± 1.66	18± 1.44

The results are expressed in ± S.E.M, at P ≤ 0.05, * Significant, **moderate significant, ***highly significant

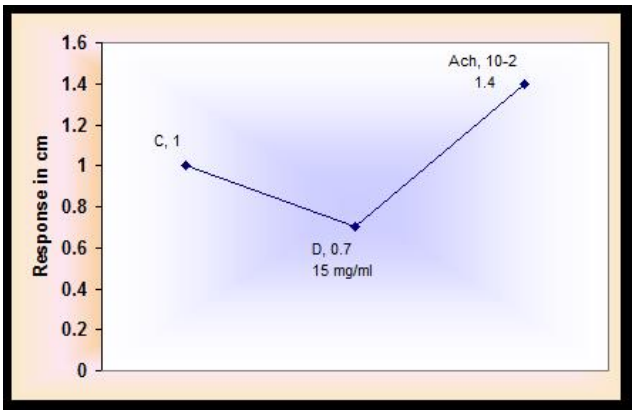
Table 6: Assessment of anti-inflammatory activity (carrageenan induced inflammation)

Treatment	Dose mg/kg orally	Mean diamtere of rat paw in mm ± S.E.M			% of inhibition		
		1hr	2hr	3hr	1hr	2hr	3hr
Control	0.5 ml Saline	18.4±2.67	19.5±2.57	20.3±2.11	-	-	-
S. racemosa	200 mg/kg	16±1.22	15.8±1.28	19.2±1.61	13.04	18.97	5.42
	300 mg/kg	15.2±1.29	14.1±1.23	18.5±185	17.391	27.69	8.87
	500 mg/kg	13.8±2.32*	13.2±1.44	14.2±3.22	25	32.31	30.1
Aspirin	300mg/kg	13.9± 2.16	12.5±1.34	13.5±1.24	24.5	35.9	33.5

The results are expressed in ± S.E.M, at P≤0.05, * Significant, **moderate significant, ***highly significant

DISCUSSION

The antidiarrhoeal activity of the crude extract of *S. racemosa* was performed *in vitro* on isolated rabbit intestine. The effects of the crude extract of *S. racemosa* and its ethyl-acetate, chloroform, *n*-butanol and aqueous fractions were observed through the contraction and relaxation of intestine of rabbits. Crude extract of *S. racemosa* was observed at the doses of 1, 5, 10, 15, 20 and 25mg/ml fig. 1. The effects of fractions were tested at the dose of 15mg/ml (fig. 2). While fig. 3 represents the tracing of the fractions along standard drugs included atropine, and acetylcholine in the concentrations of 1 x 10^{-2}, 1 x10^{-4} and 1 x 10^{-6} M.



Graph 7: Effect of *n*-butanol fraction of *S. racemosa*, post-treated tissue with Acetylcholine 1x10^{-2} M concentration.

The *S. racemosa* exhibited dose dependent decrease in intestinal contraction. However the maximum antidiarrhoeal response was observed in chloroform fraction (44.6%). Further studies were carried out on the fractions of crude extract of *S. racemosa*. By observing these data it was found that the relaxing effect of rabbit intestine that appeared in the ethyl-acetate and chloroform fraction was also appeared in crude extract, but the other two fractions does not have the effects like crude extract. This is due to the separation of chemical constituents of the drug. The result of these data also indicates that crude extract of the *S. racemosa* decreases the motility of intestine therefore it has valuable importance in the treatment of intestinal disorders such as anti-diarrhoeal agent. The fractions of the extract also showed only relaxation of isolated tissue. The standard drugs along fractions were tested for further screening of the fractions of *S. racemosa*.

The effect of ethyl-acetate fraction of *S. racemosa* on pretreated tissue with atropine 1 x 10^{-2}M, indicated the cumulative relaxing response of isolated tissue of rabbit intestine (Graph 3). When ethyl-acetate fraction administered to tissue, it decreases the response of tissue and produces the relaxation of intestinal smooth muscle. This effect almost disappeared after administration of acetylcholine 1 x 10^{-2} M, but the full contractile response of acetylcholine was not produced due to the occupancy of muscarinic receptors. In the reverse manner first tissue is treated with acetylcholine 1 x 10^{-2} then with drug, it decreased the response of tissue same as when atropine was given after acetylcholine. It can be suggested that the effect of the drug may be produced through muscarinic receptors.

Similarly the effect of chloroform fraction of *S. racemosa* pretreated and post treated tissue with atropine 1 x 10^{-2}M showed the cumulative response of the drug. This synergistic effect of the drug can help in various disorders of the gastrointestinal tract. Aqueous fraction of *S. racemosa* also exhibited the same response (Graph 6). In this acetylcholine produces its full response, which indicated that aqueous fraction of *S. racemosa* does not have any prominent activity. *n*-butanol fraction of *S. racemosa* post treated with acetylcholine 1 x 10^{-2} M has relaxing response on isolated tissue whereas acetylcholine 1 x 10^{-2} M did not produces its full response due to the blocking of muscarinic receptors.

The overall effect of the crude extract and its fractions causes a relaxation of isolated smooth muscle of rabbit intestine. Chloroform (44.6%) and *n*-butanol (41.23%) fraction of ethanolic extract of bark showed maximum decreased in intestinal activity.

Crude extract of *S. racemosa* exhibited mild to moderate analgesic and anti-inflammatory response at 500 mg/kg. Literature also confirms that bark of *S. racemosa* contains those chemical constituents that produced analgesic and anti-inflammatory effect (Sharma *et al*., 2013).

Different studies also reported that *S. racemosa* inhibited the activity of an enzyme phosphodiestrase-1 (Gomes *et al*., 2010, Abbasi *et al*., 2004), which has a significant role in many physiological function of body (Jeon *et al*., 2005). This can support the analgesic and anti-inflammatory effect. *S. racemosa* barks extract showed hepato-protective and antioxidant effect which suggests its safe use as pain killing agent. Moreover the ethanolic extract and fractions of *S. racemosa* have decreased in intestinal motility (antidiarrhoeal effect) in this study and anti-ulcerogenic activity (Krishna *et al*., 2013) provides an additional benefit in gastrointestinal disease where painkiller is necessary to use.

CONCLUSION

Due to significant analgesic, anti-inflammatory and antidiarrhoeal effect *S. racemosa* crude extract and fractions have a beneficial role in the gastrointestinal tract disorders and can be used as an antidiarrhoeal agent.

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