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Antinociceptive and Cytotoxic Effect of Extracts of *Croton bonplandianus* Leaves

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ABSTRACT

Role of phytochemicals and plant based drugs in curtailment of diseases has already been established. *Croton bonplandianus* plant has found to be popular in the healthcare system for treatment of various complaints. In the present study, plant leaves were screened for analgesic and cytotoxic activities using animal models for the safe and effective utilization of plant material. The leaves were soaked in methanol and successively fractionated using n-hexane, chloroform, ethyl acetate and water. Mentioned biological activities were determined in different groups of wistar strain mice by administering various leaves extracts in differential dose pattern (81, 54 and 27 mg/kg). Cytotoxic activity was assessed by the Brine shrimp bioassay method. Results showed that extracts significantly responded as analgesic drug comparable to standard drug using different methods of evaluation for analgesic activity. Brine shrimp bioassay in different leaves extracts also showed similar effects likewise to the standard drug vincristine sulphate. All extracts exhibited marked cytotoxic activity as well against the *Artemia salina* (brine shrimp eggs). Based on the findings, it is concluded that the leaves extracts of the *C. bonplandianus* have potent analgesic and cytotoxic effects but in higher doses only. However; phytochemical screening and isolation of active constituents will need to be explored further to endorse the utilization of the *Croton* plant clinically for various therapeutic activities.

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HY and HA presented the concept, conducted experiments and did validation. HY, HA, KF, RB and SZ wrote the manuscript. KF, RB and SZ performed data analysis and compilation, and reviewed of manuscript. SM supervised the research project.

Key words
Extract of leaves of *Croton bonplandianus*, Analgesic activity, Brine shrimp bioassay, Wistar strain mice, *Artemia salina*

INTRODUCTION

Plant based products additionally to food are also being employed as alternative medicine. Various species of plant kingdom are still required for evaluation to establish the therapeutic potential of active constituents (Danish *et al*., 2020; Feng *et al*., 2017). It has been documented that more than 200 natural based compounds are prescription medicines widely obtained from higher plants, minerals, fungi and marine sources (Sandberg and Corrigan, 2001; Füllbeck et al., 2006). Compared with modern medicine, therapeutic agents derived from plants stand to be safe owing to fewer side effects (Yasin *et al*., 2019; Calixto, 2000). Herbal medicines are growing and now currently focused as alternative medicines for preventing and curing different ailments (Grundmann and Yoon, 2014). Herbal plants are the major sources of various secondary metabolites including alkaloids, flavonoids, terpenoids, tannins, providing a significant role in the treatment of different disorders especially against microbial infections (Evans, 2002). Unfortunately, beyond many benefits of natural drugs, the efficacy and potency of medicinally valuable plants are not still discovered. Only a small percentage of plants and their species are exposed for phytochemical and fractionation screening (Malesh and Satish, 2008). Phytochemical components like flavonoids and tannins also reported for antibacterial, antioxidant, anticancer, anti-inflammatory and analgesic activity (Kumar *et al*., 2008; Jaganath and Crozier, 2010; Malan and Roux, 1979).

The genus *Croton* of the family Euphorbiaceae is documented to contain approximately 1300 species of the plants distributed in tropical and subtropical areas of the world. Many of *Croton* species possessed therapeutically

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valuable moieties. Previous studies have proven the role of Euphorbiaceae family as folk and traditional medicines. These *Croton* trees, herbs and shrubs are utilized for cardiac diseases, liver complaint, anti-pyretic, anti-inflammatory chest ailments, cancer, diabetes, arthritis and skin infections (Islam *et al*., 2019; Xu *et al*., 2018). *Croton bonplandianum*, is one of the recently identified species, documented to possess remarkable antimicrobial activity and consider to be capable to treat urinary tract infections (Ghosh *et al*., 2018). Investigations were conducted on *C. bonplandianus* stem bark for the identification and isolation of therapeutic compounds using standard protocol and analytical methods. Total flavonoids, alkaloids, phenolic contents, saponins and tannins were identified. Vitamins such as ascorbic acid (Vitamin C) and riboflavin (Vitamin B12) were also found to be present in rich quantities (Dutta *et al*., 2014; Lowry *et al*., 1951; Athanasiadis and Moral, 2013). Moreover, comprehensive evaluation on stem latex of *C. bonplandianus* showed anti-microbial and antiseptic responses upon the application on fresh wound (Sriwastava *et al*., 2010; Qureshi *et al*., 2012). Phytopharmacological analysis indicated that the leaves of *C. bonplandianus* significantly exhibited anti-inflammatory activity (Yasin *et al*., 2021), wound healing effect, owing the existence of antioxidant enzymes and rutin (Divya *et al*., 2011).

The current study is designed to examine the analgesic and cytotoxic activities of *C. bonplandianus* plant as limited data is available on this species of the plant. Leaves extracts will be prepared in methanol and then fractionation will be performed using different solvents including n-hexane, chloroform, ethyl acetate and water. For analgesic activity, animals will receive different leaves extracts (methanolic, aqueous and ethyl acetate) in different doses (81, 54 and 27 mg/kg) and so grouped accordingly. Analgesia will be observed on animal models by inhibition of writhing induced by acetic acid, formalin induced paw licking method and hot plate-induced nociceptive activity in mice. Cytotoxic activity of all leaves extracts obtained in (methanolic, n-hexane, chloroform, ethyl acetate and water) will be assessed using reliable technique of Brine Shrimp Bioassay against the *Artemia salina* (brine shrimp) eggs.

MATERIALS AND METHODS

Hexane (Sigma-Aldrich), chloroform (Sigma-Aldrich), methanol (Sigma-Aldrich), ethyl acetate (Sigma-Aldrich), distilled water and Whatman filter paper for extraction of plant. Acetyl salicylic acid (ASA) (100mg/kg/body weight)**,** acetic acid (0.8%)**,** formalin (1%), NaCl (0.9%), analgesia meter hot plate (Model EH-01 Orchid Scientific), feeding tubes, distilled water and stopwatch for analgesic activity, *Artemia salina* Leach. (brine eggs), Sea salt (NaCI), vincristine sulphate, lamp (to attract shrimps), small tank, pipettes (5, 10ml), micropipette (5-50nl and 10-100µl), glass vials, magnifying glass for cytotoxic activity were used for experiments.

Extraction of C. bonplandianus *plant*

Leaves of *C. bonplandianus* (Euphorbiaceae) were collected from University of Karachi. The plant was identified during June 2018 by the Department of Botany (Taxonomy), University of Karachi, Pakistan. A voucher specimen no. 03 was deposited in the Department of Pharmacognosy, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University Karachi, Pakistan.

The dried leaves of *C. bonplandianus* were weighed (5 Kg) and percolated for 15 days in 10 L methanol, percolation was continued till the leaves were practically exhausted (three soaking and percolation cycles). Menstrum was evaporated under reduced pressure (40ppm) and temperature of 40°C. Fractionation was then carried out for the isolation of active drug components using different solvents including n-hexane, chloroform, ethyl acetate and water. Methanolic (MeOH), ethyl acetate (EtOAc) and aqueous extract (H_2O) of *C. bonplandianus* leaves were selected for determination of analgesic activities to avoid any toxicity (Pereira *et al*., 2020; Umoh *et al*., 2020) while all leaves extracts were subjected to evaluate the cytotoxic activity through brine shrimp bioassay. These extracts were administered in doses of 27, 54, 81 mg/kg. Dose protocol was selected on the basis of previously reported studies (Okokon *et al*., 2006).

Animals for analgesic activity

Male albino mice of either sex (25-30 g) were selected for analgesic activity due to resemblance with human system. Mice were purchased from the animal house of Baqai Institute of Pharmaceutical Sciences (BIPS), Baqai Medical University, Karachi. Albino mice were kept in the standard environment and balance diet with water in animal house for 24 h at 28°C±5 temperature. Analgesic effect was evaluated through acetic acid induce writhing in mice, formalin induce liking and thermal induction by hot plate method.

Induction writhing in mice

Albino mice were kept fasted with access of distilled water for 24 h prior to the treatment. Different leaves extracts of the *C. bonplandianus* were administered orally to adult albino mice, divided into 11 groups. The control group received only distilled water (10ml/kg) while remaining three groups received MeOH, EtOAC and H_2O leaves extracts. These three groups ML (MeOH),

AqL (H_2O) and EAC (EtOAC) were further categorized in to sub groups (5 mice in each) and treated with doses of 27, 54 and 81mg/kg *C. bonplandianus* orally. Acetyl salicylic acid (ASA) was administered to the reference group (ASA) in a single dose of 100mg/kg. Acetic acid 0.2 ml (0.8%) was administered intra-peritoneally (i.p.) to all animal groups after 30 min of plant drug administration. Writhing movements (stretching of hind limbs along with contraction of abdominal muscles) were counted for 30 min with 10 min time intervals (Okokon and Nwafor, 2010; Nwafor and Okwuasaba, 2003; Okonkon *et al*., 2006). Antinociception were displayed as the reduction of the number of writhing movements in all groups.

Induction of paw licking in mice

Formalin induced paw licking method (Ceccarelli *et al*., 2003; Honda *et al*., 2000) was used to evaluate the analgesic activity of the extract. Adult albino mice weighed 25-30g of either sex recruited into 11 different groups of 6 mice each were used for the experiment. The animals were pretreated with *C. bonplandianus* leaves extracts (27, 54 and 81 mg/kg i.p) and ASA (100mg/kg) before administering formalin. 0.1 mi of 1% formalin solution was injected in sub planter region of right paw of mice. In control group 0.1 ml of 0.9% NaCl was injected in sub planter region of left paw of mouse. The licking response, a pain indicator, was observed for 30 min. The first phase of antinociception appeared after 5 min of formalin injection and second phase shown after 15 to 30 min of formalin subcutaneous administration expressing the neurogenic and inflammatory analgesic response (Obese *et al*., 2021).

Induction of nociceptive activity in mice

Thermal nociceptive response is helpful to determine the central analgesic activity (Shoaib *et al*., 2017). The effect of different leave extracts of *C. bonplandianus* on hot plate was investigated in mice. All animal were divided into 11 groups and each group comprised of 6 animals. First group served as control and received normal saline while standard group received ASA 100mg/kg orally. The animals were pretreated with different leave extracts of *C. bonplandianus* (27, 54 and 81 mg/kg i.p) and ASA (100mg/kg) before placing on hot plate. The mice were placed on hot plate maintained at 55°C±2°C and expression of nociception such as flicking, licking, rearing and jumping on hind limb was observed (Bouali-Benazzouz *et al*., 2021). The experiment was terminated 25 sec after placement of animal on hot plate to prevent tissue damage.

Brine shrimp lethality assay (BSLA) for C. bonplandianus

Brine shrimp (*Atremia salina* Leach.) eggs purchased from the local market were allowed to hatch to nauplii in one liter of sea water in a small tank and then transferred into another tank containing diluted fresh seawater (38 g of non-iodized sea salt dissolved in 1 L distilled water for enhancing the visibility of nauplii. Finally, 10 nauplii were separated carefully using micropipette for brine shrimp bioassay.

Each test sample (32 mg) was dissolved in 200µl of 1% DMSO and final volume of 20 ml was made from seawater. The stock solutions having concentration of 1600 µg/ml of each sample were prepared. Serial dilution with seawater of each sample was carried out from the stock solution to obtained 800, 400, 200, 100, 50, 25, 12.5, 6.25 µg/ml extracts. Afterwards, 2.5 ml of each extract was mixed with the seawater (2.5 ml) already containing 10 nauplii for evaluation.

Low concentrations of vincristine sulphate (10, 5, 1, 0.5, 0.25, 0.125 and 0.06 µg/ml) were prepared as positive control. For negative control DMSO (50 µl) added to already marked three of each test tubes having seawater 4.95 ml along with 10 nauphii (Asaduzzaman *et al*., 2015). All test tubes were examined after 24 h against black background using magnifying glass. Total numbers of lived nauplii were counted and percentage of lethality of brine shrimp nauplii in each test tube was computed.

The lethal concentration (LC) for 50% mortality after 24 h of exposure and the chronic LC_{50} was determined using the probit method. LC_{50} values obtained at greater than 800 ppm for plant extracts would not be considered. The extent of lethality was found to be directly proportional to the concentration of the extract. The LC_{50} (median lethal concentration) values were calculated using the regression line analysis obtained by plotting the drug concentrations at x-axis (independent parameter) and percent death at Y-axis (dependent). LC_{50} values were measured finally through a probit scale.

Abbot's expression was used to determine mortality (Abott, 1925).

$$Pt= [(Po-Pc)/ (100-Pc)] \times 100$$

Where Po is Observed mortality; Pc is control mortality.

Statistical analysis

The quantitative data were presented as mean ± SEM (standard error of mean) and SPSS (social statistical package) version 21 software was used to analyze the results. One way analysis of variance (ANOVA) with post-hoc Tukey HSD was applied to assess the difference among various treatment, standard and control groups. The computation was made at 95% of confidence interval and $P < 0.05$ was considered to be significant during comparative analysis.

RESULTS

Effect of plant extract writhing in mice

In the present study different leaves extract (MeOH, Aqueous, EtOAC) of the *C. bonplandianus* (Dose 27, 54 and 81 mg/ kg) were selected to determine the analgesic potential in acetic acid induced writhes in albino mice against standard drug acetyl salicylic acid (ASA, 100 mg/kg). IP administration of acetic acid produces pain sensation in mice characterized by writhing and abdominal contraction. The total numbers of writhes were counted for 30 min with specified time intervals and result was expressed for total number of writhes as mean±SEM (Table I).

All extracts were compared with control group and significant dose dependent reduction was noticed in acetic acid induced stretching of limbs and abdominal contraction. Analgesic activity was observed in all extracts in all doses (27-81 mg/kg) when compared with control group. Significant differences were also shown by lower *P-value* ranged between 0.05 to 0.001. However, all extracts induced enhanced analgesia in dose dependent manner even better than ASA except ethyl acetate extract (Table I).

Effect of plant extract licking activity mice

Outcomes of formalin induce liking effect against the methanolic, aqueous and ethyl acetate extracts were displayed in Table II. Results were compared with the standard drug. The significant levels were expressed by lower *P-value* ranged between 0.05 to 0.001. The (MeOH) and aqueous extracts possess significant result in high doses while EtOAC extract showed non- significant results.

Table I. Analgesic activity of different extracts of *Croton bonplandianus.*

Treatment groups	Number of writhing/ 10 min		
	0-10 min	10-20 min	20-30 min
Control	18.66±0.33	12 ± 0.55	11.33 ± 0.33
ML-27	6.66±0.33**	6.33 ± 0.33	1.66 ± 0.33***
ML-54	6.66±0.33**	3.33 ± 0.33*	0.33 ± 0.33***
ML-81	9.66±0.88*	6.33 ± 0.33**	2.33 ± 0.33***
AqL-27	10.33±0.33*	6.66 ± 0.33**	3.33 ± 0.33**
AqL-54	11.66±0.88*	6.33 ± 0.33*	5.66 ± 0.33*
AqL-81	10.33±0.88*	5.33 ± 0.33**	0.33 ± 0.33***
EAL-27	10.33±0.88	8.33 ± 0.33*	5 ±0.57 *
EAL- 54	12.66±0.88*	10.33± 0.33*	6.66 ± 0.33*
EAL-81	5.66±0.33	4.33 ± 0.33	4.66 ± 0.33
ASA	18.66±0.33*	4.0 ±0.55*	3.33 ± 0.33**

Where ML, methanolic leaves extract of *C. bonplandianus* (81, 54 and 27 mg/kg); Aq L, aqueous leaves extract of *C. bonplandianus* (81, 54 and 27mg/kg); EAL, ethyl acetate leaves extract of *C. bonplandianus* (81, 54 and 27 mg/kg); ASA, acetyl salisylic acid (100mg/kg). Data is subjected to one way ANOVA using SPSS version 21 and illustrated as mean ± SEM. Significant at $^{*}P < 0.05$, $^{***}P < 0.01$, $^{***}P < 0.001$ when compared to control (n = 6).

Effect of plant extract on nociceptive activity

Table III shows the effect of different leave extract on analgesic activity. Pretreated mice (27, 54, 81 mg/kg) revealed the dose-dependent increase in latency response in methanolic and aqueous extracts while the group that

Table II. Effect of *Croton bonplandianus* extract on formalin-induced hind paw licking in mice.

Treatment groups	Time intervals (min)					
	5	10	15	20	25	30
Control	41.56±0.71	18.24±0.64	15. ± 0.36	12.49±0.21	9.78±0.22	7± 1.05
ML-27	30±0.68	10.43±0.18*	8.49±0.65 *	7.52±0.17	5.38±0.36*	2.84±0.63 *
ML-54	28.02±0.11**	8.27±0.63*	7.69±0.31***	5.58±0.22**	4.47±0.07*	3.94±0.17***
ML-81	21.78±0.04*	6.21±0.51**	4.52±0.91***	3.24±0.81*	2.12±0.07**	2.07±0.61***
AqL-27	28.30±0.85	7.59± .47*	6.14±0.94 *	5.05±0.08	4.87±0.21*	2.23±0.11 *
AqL-54	26.54±0.54*	6.56±0.13*	5.07±0.74*	4.32±0.43*	3.67±0.04*	2.25±0.84*
AqL-81	23.41±0.71*	7.63±0.13**	5.44±0.90***	4.44±0.32*	3.87±0.42**	2.87±0.21***
EAL-27	34±0.36	7.95± 0.21*	6.54 ±0.25 *	5.89±0.87	4.15± 0.25*	3.14 ±0.79 *
EAL- 54	30.04±0.54*	8.74 ± 0.18*	5.81±0.61*	4.23±0.53*	4.09±0.27*	3.41±0.45*
EAL-81	25.66±0.71*	6.11±0.13**	5.15±0.24***	4.54±0.36*	3.34±0.61**	3.10±0.41***
ASA	8.66±0.23*	3.40 ±0.15*	2.93 ± 0.21**	2.25±0.18**	1.19±0.1.18**	0.00

For details of treatment groups, see Table I.

Table III. Analgesic activity of *Croton bonplandianus* determined by hotplate method.

Treatment groups	Mean analgesic activity (time intervals in min)				
	0	30	60	90	120
Control	7.21±0.42	7.21±0.42	7.21±0.42	7.21±0.42	7.21±0.42
ML-27	8.01±0.54	10.23±0.74*	11.11±0.15*	11.97±0.65**	12.13±0.91***
ML-54	7.99±0.94	9.61±0.21	10.70±0.48*	11.60±0.33***	12.65±0.12***
ML-81	8.86±0.62	10.82±0.36	11.09±0.66*	12.74±0.11***	13.53±0.89***
AqL-27	7.40±0.17	9.54±0.27*	10.61±0.91***	10.78±0.21*	11.80±0.87**
AqL-54	8.44±0.65	10.14±0.41*	10.60±0.29*	11.40±0.92**	12.08±0.43***
AqL-81	9.50±1.17	11.21±0.40*	11.60±.0.39***	12.41±1.73***	12.88±0.58**
EAL-27	8.00±0.64	8.20±1.00	8.67±0.63	9.02±0.89	9.30±0.14
EAL- 54	7.14±0.64	8.09±1.30	9.20±0.88	9.49±1.25	9.87±0.56
EAL-81	8.13±0.77	8.67±1.24	8.24±0.84	8.80±0.15	9.17±0.82
ASA	9.90±0.78	10.00±1.02*	11.60±0.92***	12.60±0.92***	13.44±0.78***

For details of treatment groups, see Table I.

received ethyl acetate extract of *C. bonplandianus* expressed insignificant response in all doses. Outcomes of all treated group were compared with standard group i.e., ASA (aspirin 100mg/kg) at 0, 30, 60, 90 and 120 min. The significance level expressed in term of P-value ranged between 0.05 to 0.001. The significance level was calculated with respect to the control group. The higher doses of both the extracts showed comparable significant with standard drug.

Literature supports the analgesic potential of alkaloids, flavonoids and phytosteroids including stigmasterols, campesterol and β-sitosterol. *C. bonplandianus* may contain moderate to high amount of anyone or/and all of these constituents in combination or in free form, possibly contributing towards potent analgesic effects.

Cytotoxicity of plant extract

The hexane fraction of the leaves showed comparatively moderate toxicity with LC_{50} value of 303.78 µg/ml contrawise, anticancer drug vincristine sulphate displayed LC_{50} value of 1.974µg/ml. Other extracts exhibited low cytotoxicity to brine shrimp nauplii (Table IV). High toxicity of hexane extract reflects the higher extraction, probably of alkaloidal components. On the basis of findings, cytotoxic potential of all extracts in descending order is as: VS > HL > ML > ChL > MB > EAL > AqL (Asaduzzaman *et al*., 2015).

Aqueous extract was virtually non-toxic to nauplii as exhibited very low toxicity with LC_{50} values greater than100µg/ml. On the basis of the mentioned outcomes (Table IV) the plant extracts considered to be safe and non-toxic and may be used as medicine in future after further screening.

DISCUSSION

Naturally isolated compounds such as flavonoids, tannins, alkaloids, steroids and glycosides possess analgesic activity (Sengupta *et al*., 2012; Khan *et al*., 2020; Peres *et al*., 1998). Euphorbiaceous family comprised of almost 66 genera and more than 550 species, majorities of species not only been accepted to be medicine but also utilized to treat variety of diseases globally (Islam *et al*., 2019b). *C. bonplandianus* is known to have antimicrobial, antibacterial, antifungal and wound healing potential (Dutta and Chaudhuri, 2018). Examination of the bark extract of *C. urucurana* showed the analgesic activity due to the presence of different steroids such as campesterol, beta-sitosterol and stigmasiterol showed (Peres *et al*., 1998). Methanolic leaf extract of *C. lobatus* possessed significant analgesic and anti-inflammatory activity therefore, plant extract is recommended for the management of pain and inflammatory condition (Anafi *et al*., 2017).

All the three (methanolic, ethyl acetate and aqueous leaves extracts) preparations of the *C. bonplandianus* showed significant ($p < 0.05$-0.001) dose dependent analgesic action compared with control group and standard drug acetyl salicylic acid group as marked reduction in acetic acid induce writhes and abdominal contraction was observed. On the basis of these finding, it is concluded that *C. bonplandianus* may also be utilized as an analgesic drug. In future fractionation and isolation of pharmacologically effective natural compounds will provide more safer, potent and effective drug in comparison to synthetic products with higher side effects.

Table IV. Brine shrimp bioassay of different extracts of *Croton bonplandianus.*

Test samples	Concentration (µg/ml)	Log Conc.	Probit	% Mortality	% corrected mortality	LC50 (µg/ml)
HL	12.5	1.09691	4.16	20	11.11	303.78
	25	1.39794	4.16	20	11.11	
	50	1.69897	4.48	30	22.22	
	100	2	4.48	30	22.22	
	200	2.30103	5	50	44.44	
	400	2.6020	5	50	44.44	
	800	20903	7.33	100	100	
ChL	12.5	1.09691	3.72	10	0	337.23
	25	1.39794	3.72	10	0	
	50	1.69897	4.16	20	11.11	
	100	2	4.16	20	11.11	
	200	2.30103	5	50	44.44	
	400	2.60206	5.52	70	66.66	
	800	209030	6.28	90	88.88	
EAL	12.5	1.09691	3.72	10	0	419.03
	25	1.39794	4.16	20	11.11	
	50	1.69897	4.48	30	22.22	
	100	2	5	50	44.44	
	200	2.30103	5	50	44.44	
	400	2.60206	5.25	60	55.55	
	800	2090309	5.25	60	55.55	
AqL	12.5	1.09691	3.72	10	0	1392.22
	25	1.39794	3.72	10	0	
	50	1.69897	3.72	10	0	
	100	2	4.16	20	11.11	
	200	2.30103	4.16	20	11.11	
	400	2.60206	4.48	30	22.22	
	800	209030	4.48	30	22.22	
ML	12.5	1.09691	4.16	20	11.11	334.91
	25	1.39794	4.16	20	11.11	
	50	1.69897	4.75	30	22.22	
	100	2	5	40	33.33	
	200	2.30103	5.25	60	55.55	
	400	2.60206	5.84	60	55.55	
	800	209039	7.33	70	66.66	
VS	0.06	-102218	3.72	10	0	1.974
	0.125	-0.9030	4.16	20	11.11	
	0.25	-0.6020	4.48	30	22.22	
	0.5	-0.3010	5	50	44.44	
	1	0	5.52	70	66.66	
	5	0.69897	6.28	100	100	
	10	1	7.33	100	100	

Where extracts of leaves of *C. bonplandianus* i.e HL, hexane fraction; ChL, chloroform fraction; EAL, fraction; AqL, aqueous fraction; ML, methanolic fraction; VS, vincristine.

The outcomes obtained in present investigation showed the different leaves extracts gives positive responses in reduction of writhes induced by acetic acid and marked reduction in paw licking after formalin induction. Correspondingly the pretreated mice with different leaves extract also delayed the reaction time to heat stimulus. Methanolic and aqueous extract displayed dose dependent response while the mice treated with ethyl acetate were shown nonsignif8cant result. As the methanolic and aqueous extracts of this plant also be reported that they produced anti-inflammatory response in rats (Yasin *et al*., 2021). It has been reported that writhing reflex produced by acetic acid is may be due to activation of chemo sensitive nociceptors. Acetic acid affected an increase vasodilation and permeability of blood vessels (Gong *et al*., 2019). Neurogenic and inflammatory pain associated with the formalin induction which can be repented by paw licking of the animal (Chatla *et al*., 2019). Several studies revealed that flavonoids are considered to produce inhibitory response against inflammation. Flavonoids acts as natural antioxidant used to various ailment especially obesity, cancer, gout, diabetes etc. (Nile *et al*., 2018). The analgesic activity of this plant correlates with paracetamol. As previously reported data proven that the paracetamol inhibit acetic acid induced writhing. Paracetamol showed inhibitory effect on the synthesis of prostaglandin in the brain (Saliba *et al*., 2017).

Furthermore, the extract may acquire the typical model of the drug that are centrally acting, as shown inhibitory activity for both early and late phases against formalin induce paw licking (John-Africa *et al*., 2020). Similarity with the drugs like morphine and opioid with the extract may be due to exhibit central action by increasing reaction time to heat. Hence it might be possible that the plant extracts possess the inhibition of release and activity of inflammatory mediators and/or nociceptive fibers (Mehanna *et al*., 2018).

The alcoholic extract of *C. floribundus* stem bark exhibited significant antioxidant, anticholinesterase, cytotoxic and antimicrobial activities (Barth *et al*., 2018). Antimicrobial effect was observed in aqueous and methanolic stem bark extracts of *C. megalocarpus* against certain microbial strains. Moreover, extracts were found to be safe and free from acute oral toxicity upon administration in Wistar rats. Cytotoxicity of the stem bark extract was also determined by brine shrimp nauplii (Kathare *et al*., 2021). In the present investigation HL, ChL, EAL, Aq L, and ML were subjected to lethality bioassay (Table I). It was found that upon exposure of different doses, varying degree of lethality was induced. The LC_{50} was computed through regression analysis by plotting the graph between percentages of nauplii killed

against the concentration of extracts. It was observed that the rate of mortality increases gradually with increase in test sample concentration, indicating that the degree of lethality is directly proportional to the concentration from minimum (12.5 µg/ml) to maximum (800µg/ml). Based on LC_{50} it can be concluded that all plant extracts possess mild to moderate cytotoxic activity. Henceforth, the leaves drug extracts are considered to be safe and non-toxic. Extracts may be used in clinical settings for the treatment of various ailments in future after further screening and isolation.

CONCLUSION

Plants belong to *Croton* genus of family Euphorbiaceae are utilized as folk and traditional medicines all over the world. *Croton bonplandianus* is one of the valuable medicinal plant, however little has been explored in biological activities. Leaves extracts of *C. bonplandianus* possess comparable/ higher analgesic activity in wistar mice. Qualitative analysis revealed the presence of alkaloids, phenols, flavonoids and steroids in plant extracts of *C. bonplandianus*. Presence of alkaloids and steroids are presumed for the cytotoxic activity of plant (Dey *et al*., 2019). Flavonoids and phenolic compounds are antioxidant in nature and might be responsible to control the growth of abnormal cells and so also be adjunct in anticancer therapy.

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IRB approval

This study was conducted in Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan, the IRB Approval is under the Ethical Committee of Baqai Medical University, Karachi, Ref#BUU-EC/2018/01.

Ethical approval

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Statement of conflict of interest

The authors have declared no conflict of interest.

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