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RESEARCH ARTICLE
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A COMPREHENSIVE PHARMACOLOGICAL EXPLORATION AND ANTI-CANCER PROSPECTIVE OF STEM BARK OF *CROTON BONPLANDIANUS*

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Abstract
Plants are the ultimate inevitability of planet and for the mankind and animal kingdom. Herbal plant not only source of nutrition however used as remedy from ancient period. The main goal of this study is to provide a platform for researchers to examine the potential health benefits of plant extracts across a range of illness states. The bark of the plant *Croton bonplandianus* were selected for this research study. Different pharmacological activities such as analgesic, anti-inflammatory, antifungal, antibacterial, anticancer, hepatoprotective and nephroprotective activities were performed followed by histopathological studies and brine shrimp bioassay to evaluate the therapeutic and toxic potential of different extracts of bark. The histopathological screening was reported to strengthen the pharmacological studies regarding the safety profile of extracts of the bark of the *Croton bonplandianus*. The results revealed that the various plant extracts were shown significant dose dependent analgesic activity and potentially nephroprotective and hepatoprotective may be due to the presence of antioxidant flavonoids and phytosteroides. Three distinct cancer cell lines were selected for estimation of cytotoxic activity and the results shown mild to moderate activity. Antibacterial and antifungal activities were also performed by MABA assay and agar tube dilution method respectively. Aforementioned research opens an unlimited access for the investigation of therapeutically active constituents, isolation of pure compounds and preclinical and clinical studies. Furthermore, extensive research in the several directions must be escorted to discover the new drug molecules that may be used as a raw material in the pharmaceutical industries.

Keyword: *Croton bonplandianus*, bark extract, Pharmacological analysis, anti-cancer potential, Male albino mice, Male albino rats wistar strain

INTRODUCTION

Traditional therapies play foremost part for the utilization of natural module as a medicine. It has been recorded that above 200.00 compounds from all over the world are based on various natural sources of medicines including higher plants, minerals, fungi and marine source [1, 2]. Based on new drug development limited research has been accompanied related to potency and efficacy therapeutically active plants. Only small percentage of plants and their species are exposed for phytochemical and fractionation screening [3]. In recent scenario ecological and biodegradable plant materials are the leading emphasis for the utilization and prevention of various disorders. Demands of medicinally valuable plants have been raised day by day due to safety profile against the unwanted effects of synthetic chemical produce [4-6]. Among all families, Euphorbiaceae family is one of the most important having several medicinally valuable plants. Plants these family contains various chemical constituents including diterpenoids, terpenoids, alkaloids, steroids, tannins, flavonoids, fatty acid ester, saponins and others that possessed pharmacological activities [7]. More than 700 species of *Croton* belongs to the family Euphorbiaceae have been found in tropical region, 50 are native from Africa and almost 10 species found in southern Africa [8, 9]. *C. zambesicus* Muell widely distributed in tropical Africa, a small shrub with 10m height [10]. Pleasant lavender also obtained from the leaves and used as perfumes [11].

It has been reported that several species of *Croton* concerned with carcinogenesis are also utilized as counter irritant for the treatment of various chest pain or abdominal pain associated with fever, headache etc. [12, 13]. Research revealed that *Croton zambesicus* was first time reported for anti-coagulant and vasodilating properties. Methanolic extract of *C. bonplandianus* at different concentrations showed activity against mosquito *Aedes aegypti* [14]. Phytochemical screening showed that one new isopimarane, two trachylobane diterpenoids (ent-18-hydroxy-trachyloban-3-one, ent-trachyloban-3-one), stigmasterol, a-amyrin, b-sitosterol and trans-phytol were found in the *C. zambesicus* leaves [15-18]. *Croton bonplandianus* exerts several therapeutic actions including analgesic, anti- oxidant, antibacterial, antifungal, hepatoprotective, anti-coronary, insect repellent and wound healing effects [14, 19-28]. Moreover, the leaves extract of *C. bonplandianus* showed effective hepatoprotective activities in addition with anti-inflammatory and antioxidant properties [29].

MATERIALS

Methanolic extract of bark *Croton bonplandianus* (dose 27,54,81 mg/kg/body weight), Acetyl salisylic acid (ASA) 100mg/kg/body weight, Acetic acid (0.8%), Feeding tubes, Adult male albino rats (Wistar strain), Distilled water

METHOD FOR THE COLLECTION *CROTON BONPLANDIANUM*

The dried bark (5 kg) of *Croton bonplandianus* were cleaned and percolated for 15 day in methanol (MeOH) and repeated this procedure thrice separately. Then solvent was evaporated under reduce pressure and temperature 40 $^{\circ}$C. Furthermore, fractionation was performed. Methanolic extract of bark of *C. bonplandianus* were selected for pharmacological activity [30].

ANIMALS FOR ANALGESIC ACTIVITY

Male albino mice (25-30 g) were recruited for analgesic activity, mice was arranged from the animal house of BIPS, BMU Karachi [31, 32]. Albino mice were kept with standard environment and balance diet with water in animal house of 24 hours cycle at almost 28 °C. Analgesic effect was evaluated through acetic acid induced writhing in mice.

MATERIALS

Acetylsalicylic acid (ASA) (100mg/kg/body weight)**,** acetic acid (0.8%)**,** feeding tubes and distilled water

METHOD

MeOH extract of bark of the plant *C. bonplandianus* were selected for analgesic activity against acetic acid induced writhing in mice [32-34]. For MeOH extract of bark of *C. bonplandianus* adult albino mice were divided into 5 groups of each 5 were selected for this experiment. One group was control only treated with distilled water (10ml/kg), 3 groups treated with according to the dose of 27, 54 and 81mg/kg of *C. bonplandianus* of bark extract (MeOH). Albino mice were keep fasted with access of distilled water for 24 hours before the treatment. Control group received distilled water only (10ml/kg), MeOH were pretreated with 27, 54 and 81mg/kg *C. bonplandianus* orally accordingly. Group ASA served as reference group was received acetylsalicylic acid (ASA) 100mg/kg. Acetic acid 0.2 ml (0.8%) was administered intraperitoneally (IP) to all groups after 30 minutes (min). Writhing movements (stretching of hind limbs along with contraction of abdominal muscles) were counted for 30 min with 10 min intervals resulting from IP injection of acetic acid 0.2 ml (0.8%**)** [30, 35, 36]. Antinociception were exhibited as the reduction of the number of writhing movements in all groups [37-39].

ANIMALS FOR NEPHROPROTECTIVE AND HEPATOPROTECTIVE ACTIVITY

Albino rats wistar strain adult (200 ± 10 g) of male sex were selected for nephroprotective and hepatoprotective activity purchased from BIPS, BMU Karachi. Winter method was followed for anti-inflammatory activity [40]. Animals were kept as per standard rule given by animal house with maintained temperature 25 ± 2 °C for 12hours light/dark cycle. Animals were fed with laboratory standard balance diet and water.

EVALUATION OF NEPHROPROTECTIVE AND HEPATOPROTECTIVE EFFECTS

MATERIALS

Paracalcitriol (100 mg/kg), gentamicin (100 mg/kg), distilled water, feeding tubes

METHOD

Albino rats wistar strain were weighed and distributed in to six groups (6 in each group), to the group MB-81, MB-54, and MB-27 orally administered the bark extracts of the *C. bonplandianus* (81, 54, 27 mg/kg) daily and weighed for eight days respectively. 10ml/ kg distilled water (10 ml/kg) was administered to group C and PC-GTN, paracalcitriol (100 mg/kg) administered to group PC-GTN daily for similar time period. Gentamicin (100 mg/kg) was given (orally) simultaneously to each group except group C. At the 8^{th} day of procedure all treated rats were weighed then sacrificed with the exposure of light chloroform. Blood of all animals were collected via cardiac puncture and immediately processed for the evaluation of biochemical parameters [41, 42]. For this purpose, the serum samples of all the treated rats were collected and the standard protocol was adopted for the analysis of biochemical parameters including urea, creatinine, and uric acid. Different ions levels were also determined like sodium, chloride and potassium. Liver and kidneys of all treated rats were removed surgically and kept in 10% formaldehyde for histopathological processes and analysis [37, 43].

ANTIBACTERIAL ACTIVITY

MATERIALS

Pseudomonas aeruginosa (ATCC 10145*), Bacillus subtilis (ATCC 23857), Staphylococcus aureus (NCTC 6571), Salmonella typhi (ATCC 14028),* Escherichia *coli (ATCC 25922)*, Mueller Hinton medium, dimethyl sulfoxide (DMSO), 96 well plate, parafilm, incubator, Alamar Blue Dye, ELISA reader

METHOD

For the antibacterial activity 96 Well Plate Method were adopted. Mueller Hinton medium was used to inoculate all organisms then set inoculums to 0.5 McFarland turbidity index. For the preparation of

stock solution for all the samples dissolved in DMSO (1:1 concentration). Then media was dispensed into each well and repeated same procedure in triplicate. All compounds were added in well except in control well, after that make up the volume up to 200µl of 96 well plates. Lastly added $5x10^6$ in cells in control and test wells. Parafilm was used to sealed all plate and incubate for 20 hours. Dispensed Alamar Blue Dye in all wells and shaken for about 3 hours at 80 RPM in shaking incubator. The bacterial strains growths were showed by the dye colour changes from blue to pink. Record the absorbance using ELISA reader at 570 and 600 nm [44, 45].

ANTIFUNGAL ACTIVITY

MATERIALS

Trichophyton longifusis, Aspergillus flavus, Microsporumcanis, Fusarium solani, Candida glaberata, Amphotericin B, Miconazole, Sabouraud dextrose agar (SDA) (pH- 5.5-5.6), Glass vials, test tubes with screw capped, Micropipette (100-200 ul), Tips with tip box (Sterile), DMSO (Dimethyl sulfoxide)

METHOD

Agar tube dilution protocol was selected for in vitro antifungal bioassay. For this purpose prepared test samples 24 mg from crude extract for stock solution and pure compound 12 mg dissolved in 1 ml sterile DMSO. Sabouraud dextrose agar (SDA) selected for fungal growth and preparation of media done by mixing distilled water (32.5 gm/500 ml) at acidic pH (5.5-5.6) that contained maltose or glucose in high concentration i.e.2 %. After that steamed to dissolve all contents and dispensed in screw caps tube up to 4 ml volume and autoclave at 120-121 ° C for 15 minutes. For loading samples, all the tubes allowed to cool down to 50 ° C and 66.6 µl of compound with non-solidified SDA pipette from stock solution were loaded. Then the final concentration of crude extracts 400µg/ml and to 200µg/ml was given for the pure compounds to the media. All tubes were allowed to set in slant position at room temperature to solidify. Inoculate all tubes with fungus i.e. removed from 7 day old culture of fungus with 4mm diameter piece. Streak was employed on agar surface for non-mycelial growth while DMSO used to supplement other media with reference antifungal drugs as negative and positive control correspondingly. These tubes were kept in autoclave for incubation for at least 7 days a 28±0.5 ° C. During incubation time period examined culture two times in week. In compounds amended media growth were examined with reference to negative control and calculate growth inhibition and measuring linear growth (mm) [46, 47].

ANTICANCER ACTIVITY (HeLa)

MATRIALS

HeLa cells (Cervical Cancer), fetal bovine serum (FBS), Minimum Essential Medium Eagle, standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide), penicillin (100 IU/ml), streptomycin (75 cm^2 flasks), micro plate reader (Spectra Max plus, Molecular Devices, CA, USA),96-well plates, DMSO, petri dish, pipette, Soft- Max Pro software (Molecular Device, USA)

METHOD

For the assessment of anticancer activity of the samples 96-well-flat-bottom micro plates were utilized compared via standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric method. HeLa cells (cervical cancer) were cultured in Minimum Essential Medium Eagle and fetal bovine serum 5% (FBS), penicillin (100 IU/ml), streptomycin (75 cm^2 flasks) were supplemented then placed in 5% CO_2 then incubate at 37^{o}C. Existential grown cells were collected and haemocytometer was used for counting, after that then particular medium was added for dilution. Concentration of $6x10^4$cells/ml was prepared from cell culture and was added into 96-well plates (100 µL / well). Incubate overnight then removed medium and freshly prepared medium (200 µ) was incorporated in different concentrations (1-30µM) of the compounds. After 48 hours of the incubation period 200 µL MTT (0.5 mg/ml) were mixed in each well then incubate again for 4 hours. Later on, DMSO (100µL) introduced to all wells. Degree of MTT reduction to formazan in cells was analyzed

by micro plate reader with measurement of the absorbance (570 nm). Cytotoxic activity was noted as the concentration caused 50% growth inhibition (IC_{50}) for HeLa [48]. Soft- Max Pro software was used for further processed of the results of % inhibition.

CYTOTOXIC ACTIVITY

MATERIALS

Dulbecco's Modified Eagle Medium, 3T3 cells (mouse fibroblast), fetal bovine serum (FBS), standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide), penicillin (100 IU/ml), streptomycin (75 cm^2 flasks), 96-well plates, DMSO, petri dish, pipette, micro plate reader (Spectra Max plus, Molecular Devices, CA, USA), Soft- Max Pro software (Molecular Device, USA)

METHOD

For the evaluation of cytotoxic activity of the samples 96-well-flat-bottom micro plates was used. Results were compared with standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric method. 3T3 cells (mouse fibroblast), were cultured in Dulbecco's Modified Eagle Medium and fetal bovine serum 5% (FBS), streptomycin (75 cm^2 flasks), penicillin (100 IU/ml), were supplemented then retained in 5% CO_2 then incubate at 37^oC. Grown cells were picked and count with haemocytometer and then diluted by adding particular medium. From cell culture concentration of $6x10^4$cells/ml was prepared and added into 96-well plates (100 µL / well). Incubate overnight then free from medium and freshly prepared medium (200 µ) was again included in different concentrations (1-30µM) of the compounds. 200 µL MTT (0.5 mg/ml) were mixed after 48 hours incubation period in each well then incubate once again for at least 4 hours. After that, DMSO (100µL) supplemented to all wells. Amount of MTT reduction to formazan in cells was calculated by micro plate reader with measurement of the absorbance (570 nm). Cytotoxicity was noted as the concentration caused 50% growth inhibition (IC_{50}) for 3T3 cells [48].

BRINE SHRIMP BIOASSAY

MATERIALS

Anemia salina Leach. (Brine eggs), lamp (to attract Shrimps), sea salt (NaCI), small tank, pipettes (5, 10ml), micropipette (5-50nl and 10-100ul), glass vials, magnifying glass

METHOD

A defined protocol was employed [49]. Concisely the methanolic and aqueous bark extract of *C. bonplandianus* were assayed at 800, 400, 200, 100, 50, 25, 12.5, 6.25 µg/ml concentrations. Subsequently 2.5 ml of each extract was mixed with of seawater (2.5 ml) already containing 10 nauplii for evaluation. Test tubes were kept for 24 hours, and after counting the living nauplii in each test tube and using the formula, the percentage mortality at each concentration was calculated. The LC50 was then calculated by plotting a regression line (table 3) [37-39].

HISTOPATHOLOGICAL ANALYSIS

After rats dissection livers and kidneys of each animal were removed and fixed in buffered formalin for histopathological analysis [50, 51]. All specimens were preceded for section and staining using different concentration of ethyl alcohol and xylene for clearing the processed tissue materials. Paraplast was used to embed transparent tissues. About for microns thick section was cut off using rotary microtome. After proper cleaning and fixation, slides were stained with hematoxylin and eosin (H & E stained) [52, 53]. Then labeled and studied for various morphological parameters to observed morphological and pathological changes [54, 55].

RESULTS

Analgesic activity

Table 1. Analgesic activity of different extracts of *Croton bonplandianus*

Treatment/ Dose (mg/kg)	Time Intervals (minutes)		
	0-10 min	**10 - 20 min**	**20-30 min**
Control	17.56±0.33	13.21 ± 0.11	11.54 ± 0.66
MB-27	11±0.57*	8.33 ± 0.33**	1.33 ± 0.33***
MB-54	15.33±0.33*	3.33 ± 0.33*	2.66 ± 0.33***
MB-81	15.33±0.66*	4.33 ±0.33**	1.33 ± 0.33***
ASA	16.25±0.33*	4.22 ±0.45*	3.13 ± 0.33**

Where: ASA= acetyl salisylic acid MB = methanolic bark extract of *C. bonplandianus* (81, 54, 27 mg/kg)

Hepatoprotective and Nephroprotective activity

Table 2: Effect of aqueous extract of bark of *Croton bonplandianus* on different kidney functions

SAMPLES	Uric acid mg/dl	Sodium mEq/l	Potassium mEq/l	Chloride mEq/l	Biocarbonate mEq/l
CR	1.6±0.02	143±1.27	4.2±0.15	97±0.24	28±0.18
GNT	1±0.00	141±0.16	4.6±0.32	104±0.45	20±0.03
PC-GNT	1±0.00	143±0.42	3.9±0.47	103±0.17	22±0.4
AqB-81	1.37±0.40	143±2.65	4.4±0.23	101±0.14	25±0.47
AqB-54	1.22±0.01	142±2.24	4.9±0.54	102±0.12	26±0.43
AqB-27	1.28±0.03	140±0.13	4.3±0.18	102±0.12	25±0.12

Where; CR= control group treated with distilled water (10 ml/Kg), GNT= group treated with gentamicin, PC-GNT= group treated with paracalcitriol (100 mg/kg) with gentamicin, AqB= groups treated with Aqueous extract of Bark of *C. bonplandianus* (81, 54 and 27 mg/ kg). Not significant result obtained when compared with control P>0.05. Data expressed as mean ± SEM (n = 6) (ANOVA). *P* values < 0.05 were considered significant.

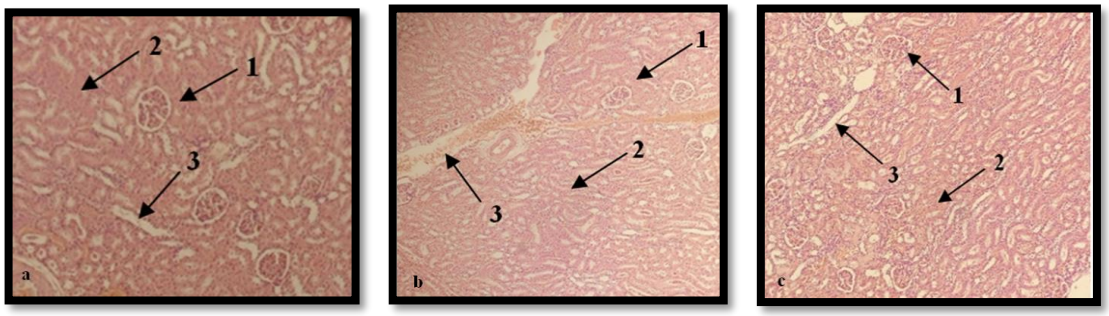

Fig. 1: Photomicrograph of 5micron thick H & E stained paraffin section from kidney rats. a: Control, b: GM, c: PC-GM.
1=Bowman's capsule, 2= Renal tubules, 3=Interstitium. a: Control, b: GM, c: PC-GM

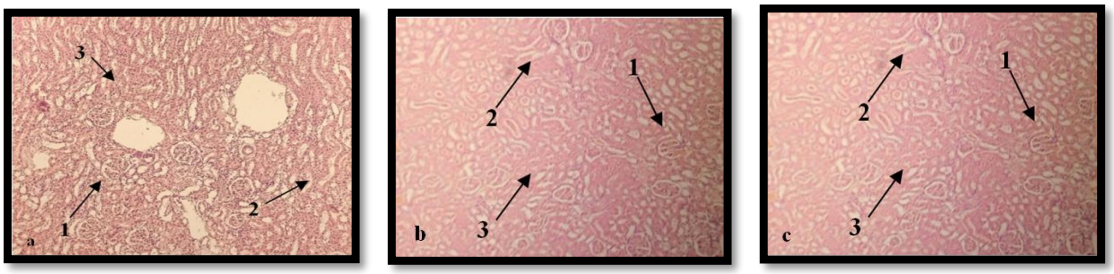

Fig.2: Photomicrograph of 5 micron thick H & E stained paraffin section from kidney rats Fig a: AqB-81, b: AqB-54, c: AqB-27
1=Bowman's capsule, 2= Renal tubules, 3=Interstitium

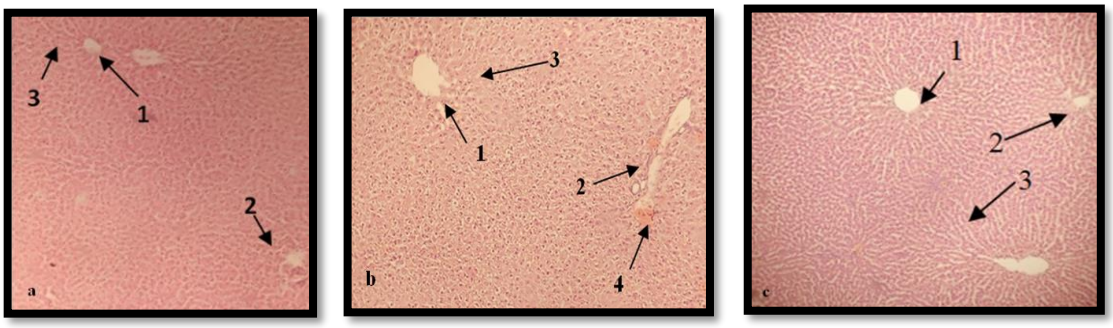

Fig. 3: Photomicrograph of 5 micron thick H & E stained paraffin section from liver of rats. Fig a; Control: 1= Central vein, 2= Portal vein, 3= Hepatic architecture. b: GM: 1= Central vein, 2= Portal triad, 3= Necrosis, 4= Hemorrhages. c: GM-PC: 1= Central vein, 2= Portal triad, 3= Hepatic architecture

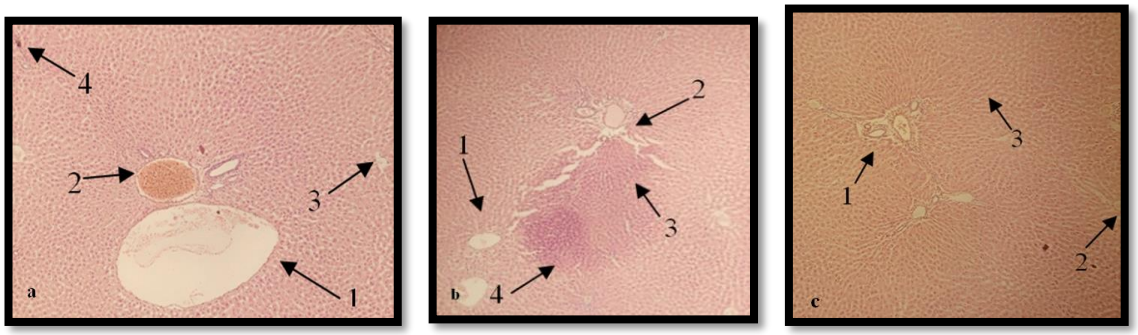

Fig.4: Photomicrograph of 5 micron thick H & E stained paraffin section from liver of rats.
Fig a: AqB-81: 1= Central vein, 2= Portal triad, 3= Sinusoid, 4= Necrosis. b: AqB-54: 1= Central vein, 2= Portal triad, 3= Hepatic architecture, 4= Necrosis. c: AqL-27: 1= Central vein, 2= Portal triad, 3= Hepatic architecture

Brine shrimp bioassay

Table 3: Brine shrimp bioassay of different extracts of *Croton bonplandianus*

Test Samples	Concentration (µg/ml)	Log Conc.	Probit	% Mortality	%Corrected Mortality	LC50 (µg/ml)
MB	12.5	1.09691	4.16	20	11.11	338.97
	25	1.39794	4.48	30	22.22	
	50	1.69897	4.75	40	33.33	
	100	2	5	50	44.44	
	200	2.30103	5.25	60	55.55	
	400	2.60206	5.25	60	55.55	
	800	209030	5.25	60	55.55	
AqB	12.5	1.09691	4.16	20	11.11	754.318
	25	1.09691	4.16	20	11.11	
	50	1.39794	4.48	30	22.22	
	100	1.39794	4.48	30	22.22	
	200	1.39794	4.48	30	22.22	
	400	1.69897	4.75	40	33.33	
	800	2	5	50	44.44	
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 MB = methanolic bark extract of *C. bonplandianus*, AqB= aqueous extract, VS= vincristine sulphate

Anti-Cancer and Cytotoxicity Avtivity

Table 4. Cytotoxic activity methanolic bark extract of *Croton bonplandianus*

Samples	Concentration	%inhibition/stimulation	IC_{50}± SD
MTT HeLa Assay			
MB	30 µg/ml	76.5	21.4±0.8
Doxorubicin	30µM	101.2	0.9±0.14
MTT (3T3) Assay			
MB	30	22.5	Inactive
Cyclohexamide	30	89.9	0.8±0.1
MTT (PC3) Assay			
MB	30	55.2	More than 50

Where; MB= methanolic bark extract of *Croton bonplandianus*

Antibacterial activity

Table 5. MABA Assay for methanolic bark extract of *Croton bonplandianus*

% inhibition of compound Sample	*Escherichia coli ATCC 25922*	*Bacillus subtilis ATCC 23857*	*Staphylococcus Aureus NCTC 6571*	*Pseudomonas aeruginosa ATCC 10145*	*Salmonella typhi ATCC 14028*
MB	No inhibition	2.74	No inhibition	6.85	No inhibition
Standard drug	84.23	88.69	84.12	85.93	88.43

Where; MB= methanolic bark extract of *Croton bonplandianus*

Table 6. Antifungal activity methanolic bark extract of *Croton bonplandianus*

Name of fungus	ML Methanolic bark extract						Std. Drug	MIC (µg/mL)
	Linear growth (mm)			Linear growth (mm)				
	Sample	Control	% Inhibition	Sample	Control	% Inhibition		
Trichophyton rubrum	100	100	0	100	100	0	Miconazole	70
Aspergillus niger	100	100	0	100	100	0	Amphortericin B	20
Microsporum canis	100	100	0	100	100	0	Miconazole	98.4
Fusarium Ini	100	100	0	100	100	0	Miconazole	73.25
Candida glabarata	100	100	0	100	100	0	Miconazole	110.8
Aspergillus fumigatus	100	100	0	100	100	0	Amphortericin B	100

DISCUSSION

Analgesic activity

Investigation supported that the *Croton bonplandianus* have potential activity against several infectious diseases and numerous injuries [29]. MB group was acquired significant (P< 0.05-0.001) dose dependent analgesic activity compared with the control group and standard drug acetyl salicylic acid with marked reduction in acetic acid induced writhes and abdominal contraction (Table 1).

Hepatoprotective and Nephroprotective activity

Kidney is an important organ responsible for the excretion of waste products, removal of extra fluids, water, minerals and balancing of electrolytes. Renal efficiency can be evaluated by the biochemical markers such as urea, creatinine, uric acid, sodium, chloride, potassium and bicarbonate [56]. Routine

analysis of such parameters were consolidates and confirms the kidney function [57, 58]. Insignificant changes were observed in all groups reflecting that the administration of gentamicin alone or in combination may not alter the levels of sodium, potassium, chloride and uric acid (Table 2). However, significant reductions in creatinine and urea were exhibited in MB group compared to the group that received gentamicin alone. Improvement in kidney functions is found to be nearly more or less similar in treated group (Fig 1, 2). Liver is a vital organ involved in various metabolic, secretary and excretory functions of the body. Liver injury or damage may lead towards the critical complication or even death [59]. Liver function can be examined by the SGPT, ALT, GGT etc. Results of the present study showed that bark extract was responsible to reduce the elevated level of SGPT, ALT, GGT and bilirubin significantly in all doses (27-81). The dose dependent effects of bark extract are presented in figures (Fig 3, 4).

Brine shrimp bioassay

BSCT was used for the evaluation of cytotoxic effects of plants extracts. BSCT is an effective method to correlate between the cytotoxicity and pharmacological properties of plant material [60]. In the present investigation MB and AqB extracts were subjected to lethality bioassay (Table 3). It was found that with the exposure of different dose, varying degree of lethality was observed. The LC_{50} value was calculated by regression analysis through plotting the graph between the percentages of nauplii killed against the concentration of extracts. The rate of mortality was increased gradually with an 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). From the LC_{50} it can be concluded the all extracts possess mild to moderate cytotoxic activity. On the basis of the mentioned outcomes (Table 3) the plant extracts are considered to be safe non-toxic and henceforth may be used as medicine for the treatment of various ailments in the future after further screening [61].

Cytotoxicity activity

Cancer is being considered as one the most complicated disease that may ultimately cause death globally [62]. Although extensive investigations have carried out for the development of new anticancer agents but most of the chemotherapeutic agents only improve the overall condition of patients however unable to eliminate the cancer absolutely [63, 64]. Chemotherapy is being considered as front-line treatment for various types of cancer. Drug resistance against the cancer therapy become a major problem in current scenario [65, 66]. The anticancer activity was noticed by researcher in various species of the C*roton* [67]. Cytotoxic activity in the methanolic extract of the bark was investigated. The outcomes revealed that the bark exhibited the low to significant activity (Table 4). Present study recommended that the bark of *Croton bonplandianus* exhibited cytotoxic potential. On the behalf of above outcomes it is suggested that the studies on isolated components with detail sophisticated cytotoxic analysis will be carried out for such extract. This may provide natural chemotherapeutic agents in future prospect for better treatment choice.

Antibacterial activity

Antibacterial activity of bark extract of *C. bonplandianus* was determined using MABA (Table 5). The methanolic bark extract exhibited low antibacterial activity [44, 45]. In forthcoming, other sophisticated techniques may be used with different bacterial cultures to evaluate the antibacterial activity of *C. bonplandianus*.

Antifungal activity

The antifungal activity was carried out MB by different fungal cultures against common fungal strains of *Trichophyton rubrum, Aspergillus niger, Microsporum canis, Fusarium lini, Candida glabarata* and *Aspergillus fumigates*. The zone of inhibition were presented in percentage comparison against the standard drugs Miconazole and Amphotericin B. Various species of *Croton* are reported to possess antifungal activities [68]. The ML, AqL and MB extracts showed insignificant antifungal activity

(Table 6). It was recommended for the evaluation of antifungal activity different methods with other strains of fungal culture might be selected for advance investigation in future [46, 47].

Conclusion

On the basis of above finding it is concluded that *C. bonplandianus* may be used as analgesic drug. In future fractionation and isolation of pharmacologically effective natural compounds will be prime an objective. It is concluded on the basis of above discussion that the aqueous extract of *Croton bonplandianus* has hepatoprotective and nephroprotective therapeutic potential. The plant extract have ability to reduce gentamicin induced renal and hepatic functional and structural abnormalities. Hepatotoxicity and nephrotoxicity induced by gentamicin might be reduced by antioxidant action due to the presence of flavonoids, steroids and phytosteriols. It was offered that the cytotoxic activity of plant material may be due to presences of alkaloids and steroids. However, flavonoids and phenolic compounds also possessed cytotoxic activity therefore plant extract may be used as antioxidant and anticancer drugs.

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