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Archives of Applied Science Research, 2010, 2 (2):211-216
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Evaluation of genotoxic and antimicrobial potential of *Croton bonplandianum* Baill.

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Abstract

Aqueous, acetone and methanol leaf and fruit extracts of Croton bonplandianum Baill. were tested for genotoxicity and antimicrobial activity. Treatment of Allium roots revealed that all the extracts were slightly mitodepressive in nature. Leaf extracts of the plants prepared in acetone and methanol showed significant mitodepression only in 24 h treatment sets. Further various extracts induced a variety of clastogenic and non-clastogenic aberrations in the root tip cells (RTCs). The aqueous extract (leaf) produced 24.17% aberrations in the RTCs at anaphase/telophases followed by methanol extract (22.08%) and acetone extract (21.55%). The fruit extracts are less genotoxic as there are fewer cytological aberrations produced in RTCs at A/T phases. The antimicrobial studies revealed that methanol extract of leaf and fruit of Croton bonplandianum is more effective against tested microbes than aqueous and acetone extracts. The methanol extract showed maximum activity against gram positive bacteria and acetone extract of leaves showed maximum activity against gram negative bacteria. None of the extracts showed activity against Pseudomonas aeruginosa.

Key words *Croton bonplandianum*, Genotoxicity**,** Antimicrobial activity assay**,** *Allium* assay.

INTRODUCTION

Plant based products are important therapeutic resource in the treatment of many illness. Data from world health organisation show that 70-80% of the world's population use herbal medicines as alternative medicine [1]. Medicinal plants owe their curative value to hoard of chemical substances present in various plant tissues. Plants at the same time also synthesize toxic chemicals apparently as primary defence against pathogens and predators [2]. There is considerable evidence indicating that many of the plant based products are toxic to human. Hence every herbal product needs thorough testing.

Croton bonplandianum Baill. (Family- Euphorbiaceae), a native of America, is a valuable medicinal herb. It is found throughout India and the tropical and subtropical regions of the world. The plant has been credited with potential to cure liver disorder, swelling of body, cure against ringworms and skin diseases [3]. Bark and roots are alternative and chologogue. Seeds are

purgative. The fresh juice of the plant is used against headache by ethinic groups. Latex of plants has healing effect on cuts and wounds [4, 5]. The latex of the plant (1:5 v/v in 50% acetone) showed antifungal activities by causing absolute inhibition against two ringworms fungi viz. - *Microsporiun gypsum* and *Trichophyron mentagrophyt* [6]. In this study the antimicrobial activity of water, acetone and alcohol extracts of leaves and fruits of *Croton bonplandianum* against a panel of gram positive and gram negative bacteria has been studied. These extracts have also been investigated for genotoxic activity using root tip meristem based *Allium* assay. The *Allium* assay is a widely used and popular method for screening large number of chemicals and environmental samples. The aim of present work is to determine the genotoxic and antimicrobial potential of *Croton bonplandianum* Baill. so as to minimize possible human health risks.

MATERIALS AND METHODS

Plant Material

The plants of *Croton bonplandianum* for the investigation were collected from Punjabi University Campus, Patiala. The voucher specimens are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala.

Preparation of Plant Extracts

Extracts of fresh leaves and fruits were used for genotoxicity and antimicrobial assay. Five grams fresh plant material of each (leaves or fruit) was homogenized and was extracted in 100 ml of solvents (distilled water, acetone or methanol) for two days on a rotary shaker. Extract was filtered and centrifuged at 6000 rpm for 25 min. The supernatant was collected and concentrated in a rotary evaporator. The extract then dissolved in 100 ml double distilled water or in DMSO (1:1 w/v) for genotoxicity and antimicrobial assay, respectively.

Genotoxicity Assay

Allium assay was carried out on the lines suggested by Fiskesjo [7] and later modified by Rank and Nielsen [8]. Healthy, nearly equal sized bulbs of onion (*Allium cepa*) procured from local market, were used. The outer scales of bulbs were removed in order to expose young root primordia. These bulbs were then placed on test tubes filled with water in such a way that the lower portions of bulbs were dipping in the water. The bulbs were placed in above condition for 2 days (48 h) at 20 ± 1^0C in an incubator in dark, changing water after 24 hour. The germinating bulbs with fresh healthy roots were placed on different test tubes containing, tap water (negative control), maleic hydrazide (1mg/l as positive control), aqueous extract, acetone extract, methanol extract. Three roots from each bulb were fixed in Carnoy's fixative (6 parts ethanol: 3 parts chloroform: 1 part glacial acetic acid) after 4 h and then 24 h of treatment. Whole experiment was conducted in triplicate. Mitotic preparations were made by hydrolyzing the root tips in a mixture of 1N HCl and 2% acetocarmine (1:9) at 50^0C for 5 minutes. After maceration the root tip squashes were prepared in 2% acetocarmine and observed under microscope. Each root tip meristem was scanned taking 10-15 observations at random under microscope. Out of total number of RTCs observed, the dividing cells were counted for mitotic index. The abnormal cells were classified according to the nature of aberrations.

Antimicrobial Activity Assay

The antimicrobial activity of three types of extracts of leaves and fruits was tested by disc diffusion method [9] using *Bacillus subtilis* (MTCC ACC NO 2757), *Escherichia coli* (MTCC ACC NO 3261), *Klebsiella pneumoniae* (MTCC ACC NO 3384), *Pseudomonas aeruginosa* (MTCC ACC NO 1035), *Staphylococcus aureus* (MTCC ACC NO 740/96) as test organisms.

These strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh. All bacteria were sub-cultured in nutrient broth (13g/l) at 37^0C for 24 h. All tests were carried out in triplicate.

Antimicrobial activity assay was conducted using disc diffusion method. In this method, sterilized Petri dishes were preseeded with 30 ml of agar containing growth medium and 0.5 ml of inoculum (inoculum's size 10^4 cells/ml). Sterile paper discs measured 9mm diameter that absorbed 20µl of the test sample (aqueous, acetone or methanol extract of leaves and fruits) was placed on the solidified plates under aseptic conditions and then incubated at $37^0 \pm 1^0$C for 24 h. The diameter of inhibition zone was measured and compared with those of the standard references i.e. positive control (20µl Chloramphenicol) and negative control (DMSO).

RESULTS AND DISCUSSION

Three types of extracts of leaves and fruits of *Croton bonplandianum* Bail. i.e. aqueous, acetone and methanol extracts have been investigated for and genotoxicity and antimicrobial activity. The results are presented in Tables 1, 2.

The results of the screening of crude plant extracts of *Croton bonplandianum* Baill. for genotoxic response in *Allium* assay are presented in Table 1. It was observed that all the extracts were slightly mitodepressive in nature as compared to the control. Leaf extracts of the plants prepared in acetone and methanol showed significant mitodepression only in 24 h treatment sets. Maximum mitodepression was observed in the roots treated (24 h) with acetone extract of fruits. Treatment to *Allium* roots with various extracts induced a variety of cytological aberrations. Both clastogenic and non-clastogenic aberrations were observed. Attempts have been made to analyze the aberrations at anaphases and telophases [8]. As far as the total aberrations induced in the RTCs at anaphases/telophases is concerned the aqueous extract (leaf) produced aberrations in 24.17% cells followed by methanol extract (leaf) i.e. 22.08% and acetone extract i.e. 21.55%. The fruit extract produced fewer cytological aberrations than those of by leaf extracts. The methanol extract of leaves proved to be highly toxic as it produced maximum clastogenic aberrations (bridges, laggards and micronuclei). Same is true for methanol extract of fruits. Such abnormalities have earlier been reported by many workers using plant extracts [10, 11, 12, 13, 14]. These clastogenic aberrations have been attributed to induction of break at DNA level by the treatment reagents. Production of bridges at anaphase and telophases can be attributed to breakage and reunion of chromatids or subchromatids [15]. Non-clastogenic aberrations include stickiness of chromosomes, vagrants, pycnosis and multipolarity. Aberrations in chromosomal proteins may change the surface nucleoprotein configuration resulting in stickiness or abnormal spindle activity [16].

The antimicrobial assay, using microbes, revealed that among various types of the extracts the methanol extracts of leaves and fruits were more effective against the tested microbes than aqueous extracts and acetone extracts (Table 2). The aqueous extracts exhibited minimum antibiotic activity among three types of the solvents. Similar results have been obtained by many workers on a variety of medicinal plants [17-19].

Table 1: Percentage of Mitotic index and Cytological aberrations in RTCs at anaphase and telophase of *Allium cepa* after treatment with Aqueous, Acetone and Methanol extracts of *Croton bonplandianum*.

Treatment	Total RTCs studied	Mitotic Index (%±SD)	Total cells at Anaphase & Telophase	Aberrant cells						Total Aberration (%±SD)	Total Clastogenic aberrations (%±SD)
				Br	Lag	V	Py	Mp	Mn		
Tap water											
4h	3839	34.33±3.89	258	05	06	04	--	--	--	5.81±0.38	4.26±0.27
Maleic hydrazide											
4 h	4341	29.99±1.50	212	31	19	08	08	04	05	35.37±0.43	23.54±4.03
24 h	4208	28.06±4.10	248	34	23	16	26	01	06	42.74±5.03	22.97±3.13
Leaf extract											
Aqueous											
4 h	4106	34.77±4.29	242	14	15	12	--	--	04	18.59±2.06	11.57±0.28
24 h	4071	28.12±3.26	211	15	17	12	--	03	04	24.17±3.01	15.15±0.67**
Acetone											
4 h	4005	29.88±3.70	228	17	12	15	--	--	--	19.29±1.10	12.71±1.54**
24 h	4186	29.14±1.93*	218	19	08	15	--	04	01	21.55±3.44	12.37±3.57**
Methanol											
4 h	3778	31.14±2.97	238	18	09	08	--	--	02	15.54±2.77	11.34±2.67**1
24 h	4144	29.50±2.62*	171	15	11	09	02	02	--	22.80±3.34	15.20±1.65**
Fruit extract											
Aqueous											
4 h	4316	28.84±1.01*	223	09	04	05	--	--	--	8.06±1.18	5.82±1.58
24 h	4243	29.74±1.64*	261	18	07	07	--	--	--	12.26±2.43	9.57±2.97**
Acetone											
4 h	4480	30.13±2.67	295	11	03	03	--	--	--	5.76±1.56	4.73±1.91
24 h	4345	26.9±4.24**	234	13	09	10	--	--	--	13.67±0.88	9.39±1.20
Methanol											
4 h	4020	29.15±0.95*	234	12	06	07	--	--	--	10.67±1.37	7.68±1.81
24 h	4361	28.81±1.72*	220	15	07	09	--	--	--	14.09±1.88	9.99±2.56*

Abbreviations: Br – bridges, Lag– laggards, V – vagrants, Py - pycnosis, MP – multipolarity, Mn – micronuclei.
** Significant at 0.05; ** Significant at 0.01*

Table 2: Antibacterial activity and Activity index of Aqueous, Methanol, and Acetone extracts of leaves & seeds of *Croton bonplandianum*.

Organism	Zone of inhibition (mm)								Activity index					
	CPC (+)	DMSO (-)	Leaf extract			Seed extract			Leaf extract			Seed extract		
			Aq	Ac	M	Aq	Ac	M	Aq	Ac	M	Aq	Ac	M
Bacillus subtilis	45	---	13	34	37	28	35	37	0.29	0.75	0.82	0.62	0.78	0.82
Escherichia coli	42	---	19	33	36	26	34	32	0.45	0.79	0.86	0.62	0.81	0.76
Klebsiella pneumoniae	45	---	16	35	34	13	36	38	0.36	0.77	0.76	0.29	0.80	0.84
Pseudomonas aeruginosa	23	---	00	---	---	00	---	---	00	00	00	00	00	00
Staphylococcus aureus	43	---	11	34	32	25	33	38	0.26	0.79	0.74	0.58	0.77	0.86

Abbreviations: Aq – Aqueous, Ac – Acetone, M – Methanol, CPC- Chloramphenicol, DMSO – Dimethylsulphoxide

Comparatively the fruit extracts showed more antibiotic activity than leaf extracts (Table 2). The various extracts, however, differ in their response toward different microbes. None of the extract showed any activity against *P. aeruginosa*. Interestingly, the methanolic fruit extract showed higher antibiotic activity (activity index) against all bacteria except *E. coli* in which acetone extract of fruits showed highest activity.

Determination of the safety of natural products is very important since toxicity represents an important obstacle in drug development. In the ethno-pharmacological context, the lack of toxicity and genotoxicity are important for the whole population. Biochemically, the plants of *Croton bonplandianum* are reported to contain flavonoids, saponins and alkaloids [20] rutin, phorbol derived crotsparine and crotsparinine [5]. Out of these components saponins [21] and phorbol esters [22] reported to possess antimicrobial activity. Rutin enhance the antimicrobial activities of other flavonoids [23].

CONCLUSION

The antimicrobial response and toxicity of three types of extracts of *Croton bonplandianum* can be attributed to the different constituents of the plant. Detailed biochemical analysis and testing of individual components is required to establish clearly the identity of antibacterial or genotoxic agents. The plant has the potential to be used in antibiotic drug formulations but should be avoided in oral or edible drug preparations.

Acknowledgements
The authors are grateful to Head, Department of Botany, Punjabi University, Patiala and to University Grants Commission, New Delhi (DRS and ASIST) for providing necessary facilities.

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