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STUDY OF ANTIMICROBIAL/ ANTIBIOTIC ACTIVITIES OF *LAPORTEA CRENULATA* GAUD

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Abstract: The poisonous plant *Laportea crenulata* Gaud. has been studied to find out its poisonous nature in aqueous extract of leaf, leaf sap extract, extract in petroleum ether, alcohol, chloroform, stinging hair contact with discs tested to antibiotic activity against bacteria and an ascomycetus fungus, *Saccharomyces cerevisiae* and also a phycomycetus fungus, *Aspergillus* sp.. The extract showed antibacterial activity against *Escherichia coli, Rhizobium* sp. and *Agrobacteria* sp.. The fungi *Saccharomyces cerevisiae* and *Aspergillus* were tested. Leaf sap extract showed highest inhibition of Microbial cells. *Escherichia coli* is highly sensitive to all sorts of plant extracts amongst all micro-organisms tested. There was no inhibitory action on a mycelial fugus but there was a positive action on non-mycelial fungus.

Introduction

Laportea is a member of the family Urticaceae. It has stinging hair on the lower surface (adaxial). This plant is locally known as 'Agnichutra' (khan, M.S. and Hassan, M.A. (1981). We *have* first found this plant in Rangpur. Professor Anisur Rahman (the fomer vice-chancellor of Rajshahi University) did not know this plant until 1992, while he was found shrubs nearly the circuit House in Rangpur. The fruits are attractive and looks like rosary in bunches. Curiously Professor Rahman touched the fruits but unconsciouly the skin of his hand also touched the stinging hairs of "Agnichutra". Later on he had isching effect. When he washed his hands, first a painful irritation then a burning sensation developed and he had to suffer continuously for seven days. He could not even sleep for three nights after stinging with the hairs of 'Agnichutra'. Then we thought to work on this whether the toxins present in this plant can be used for any useful purposes of man.

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This experimental plant *Laportea crenulata* is a poisonous one (chopra, Badhwar, Ghosh 1984). It has a positive effect against cells. So, that it may be used for the treatment of cancer cells, tumour ceils, itching, skin diseases and also as fungicides and bacteriocides (Bambury, R.E.1979, Islam, N and Ghori, A. 1996).

This plants may be contain alkaloids, glucosides or toxins. This phytochemical substance have antibacterial and antimicrobial activities. The plant leaves which can showed strong cytotoxic effects against cancer cells, tumour cells and effects against the skin diseases. Stinging hairs of *L. Crenulata* contain toxic substances. When injected into the skin or in epithelial tissue gives a burning sensation in the body. There fore, think, that, it can have a good use for human. We can benifited very much by this poisonous plants.

The present work reports the results of studies on the ' Agnichutra'. *Laportea crenulata.* First the leaves with the stinging hairs were dipped in water and put for 12 hours and the extracts were used for studies. Next the plant leaves were soaked in water and heated to make extract. (Alam, M.K., Rhshid, H.and Choudhury, N. 1992). Moreover, the whole twig was used to make extract in water, in alcohol, in petroleum ether, in chloroform and these were used to see antimicrobial/antibacterial activity, (Bauer, A.W., Kirby, W.M.H, Sherris, J.C. and Turck, M. 1996) if there was any. The results are presented in this thesis. So far we know, there has not been any work on this plant.

If we get active ingredient (s) or any toxin (s), this/these may be used for further investigaition against tumour cells or cancer cells as a remedial measure for human benefit.

Materials & Methods

Plant materials, laboratorical glass-ware, Chemicals used in this work were collected locally.

Microorganisms: Microorganisms were obtained from the pharmacy department and Tissue culture & breeding laboratory University of Rajshahi.

Preparation of Plant Extract

Fresh young shoots with mature leaves (200g) were prepared by taking 100 ml sterile distilled water. Then it was boiled at 100° *C.* for 5 minute (Harbrone 1976) and then cut into small pieces and well blended well. After cold of the boiled water a fine mesh cloth use for filter and then centrifuged at 1000 RPM for 10 minutes. The filtered water collection

with carefully. The next time the extract was filtrate *off* by the filter paper. (Khan et al. 1989). The clear aqueous extract (Alam, M.K., Rhshid, H. and Choudhury, N. 1992) which was subsequently used for antimicrobial study after sterilization.

Fresh mature leaves (250g) were well ground on a clean blender without water, alcohol, chloroform or petroleum ether. By using neat and clean fine mesh cloth for filter. The filterate extract then atutoclaved five minutes only. Then the extract were stored and collected with best careful (Hossain, M.S., Fertdous, A.J. and Hasan, C.M. 1993). 32 ml of leave extract which was subsequently used for experiments.

Poisoning may be produced through contact with the absorbent filter paper dise. Sterile filter paper discs were contact between leave with stinging hair. The disc was holding by forcep. The absorbent paper disc was used for antimicrobial activity (Khan et.al. 1989).

Antimicrobial Sensitivity: All inoculations and aseptic manipulations were carried out in a inoculation chamber. The inoculation chamber was switched on UV light for 15-30 minutes before used and cleaned with 90% ethyl alcohol to reduce the chance of contamination. All instruments like needle forceps, sperader, inoculating loop etc. were covered with aluminium foil and sterilized by steam sterilization method. During working time these were again sterilized by an alcoholic dip and flaming method inside the inoculation chamber. To ensure complete aseptic condition, both bands were also wiped by 90% ethyl alcohol.

The *in vitro* sensitivity of the micro-organisms to the extracts were done by the disc diffusion method (Bauer et al. 1966) using the adsorbed discs containing the test materials. Approximately 15-20ml autoclaved B.D.M. agar nutrient medium cooled down at 45°C-50°C was poured into each sterile petri-dishes and loft on the table for solidification. After solidification the plates were inoculated with microorganisms by sterile inoculating wireloop from pure stock culture and then inoculation of media was good sfreaking. The discs were prepared with different concentration of specific extract. Discs were soaked in each specific extract for 24 hrs only and dried. The test materials discs (extract/disc) were placed on the inoculated plates with sterile forcep. After inoculation the petri-dish were labelled by glass marker pen. All the agar plates were kept in the refrigerator for 2-4 hrs for the completion of diffusion. The plates were inoculated with the respective organisms and incubated in incubator for 24 hrs at 37°C and for 48 hrs at 30°C, respectively, for bacteria and fungi.

The activity was determined by measuring the diameter of the zone of inhibition (Hasan et.al. 1988). The inhibitory zone of microbes growth was measured the whole diameter with discs in mm. The sensitive zones were recorded as 0.0 mm to 12 mm; the diameter of the zone of inhibition was measured using a fine scale. The sensitivity test of microbes were done to different extracts. Total output of this study are presented in results and discussion section.

Results and Discussion

Antibacterial tests were made against five Organisms: *(I) Escherichia coli,* (2) *Rhizobium* sp. (3) *Agrobacteria* sp. (4) *saccharomyces cerevisiae* and (5) *Aspergillus* sp. The results of three Gram negative bacteria and two fungi which were tested against the poisonous extracts are presented in Tables.

Aqueous extract, leaf sap extract, petroleum ether extract, alcohol extract, chloroform extract and hair contact extracts were active against *Escherichia coli.* It is seen that leaf sap extract, extract in petroleum ether, alcohol extract, chloroform and hair contact extract were active *against Rhizobium* sp.. But only leaf sap extract was active *against Agrobacteria sp.* It is seen that aqueous extract, leaf sap extract, extract in petroleum ether and alcohol were active against *saccharomyces cerevisiae.* There was no extract which could inhibit the growth *of Aspergillus* sp..

The average diameter of the zone of inhibition was found intermediate using YEM medium. NA medium there were lowest zone of inhinition area. There fore, the media may have an influence for diffusion of poisonous substances. In YEP medium there was highest zone of inhibition, indicating that the diffusion of poisonous sunstances were higher in comparison with other media.

Sap extract of leaf was active against the four organisms tested. They are *Escherichia coli, Rhizobium* sp, *Agrobacteria* sp. and *Saccharomyces cerevisiae.*

Table 1: Results of Antimicrobial Activities of Different Extracts from *Laportea Crenulata.*

Test Organisms	Cl. Dist. Wt.	AQ. Ext. of leaf	Sap Ext. of leaf	PE. Ext. of leaf	Ale. Ext. of leaf	CF. Ext. of leaf	Hair Cont. of disc Ext.
E. coli	—	+	+	+	+	+	+
Rhizobium sp.	—	—	+	+	+	+	+
Agrobacteria sp.	—	—	+	—	—	—	—
Saccharomyces cerevvaae	—	+	+	+	+	—	—
Aspergillus sp.	—	—	—	—	—	—	—

+ = Antimicrobial positive
— = No activity

Cl. Dist Wt = Control distill water
AQ. Ext = Aqueous extract
PE. Ext = Petroleum ether extract
Ale. Ext = Alcohol extract
CF. Ext = Chloroform extract
Cont. = Contact

Table 2: Studies of Antibacterial Activities of *Laportea Crenulata* Plant Extracts on Yep Medium.

Test Organisms	Disc No.	CLDist Wt. Discs 5 /plate	Diameter of Inhibition Zone in Milimeter (mm) after 48 Hrs of Incubation					
			AQ. Ext. Discs 7 /plate	Leaf sap Ext. Discs 9 /plate	Leaf Ext. in PE Discs 5 /plate	Leaf Ext in Alc. Discs 9 /plate	Leaf Ext. in CF. Discs 6 /plate	Hair cont. of Discs 5 /plate
E. coli	1	0	12	12	8	12	10	7
	2	0	9	10	10	9	12	7
	3	0	9	12	10	9	9	8
	4	0	8	9	9	8	9	8
	5	0	7	12	12	8	9	7
	6	0	8	9		7	10	
	7		8	9		9		
	8			10		7		
	9			9		8		
AV. area inb. zone		0	8.71	10.22	9.8	8.55	9.83	7.4

The size of the paper discs was 6 mm.

AQ = Aqueous
Ext = Extract
PE = Petroleum ether
Alc = Alcohol
CF = Chloroform
Cont = Contact

Cl. Dist Wt = Control in distilled water
AV. = Average
INB = Inhibition
Zo = Zone

Aqueous extract was active against the two organisms tested. They are *Escherichia coli* and *Saccharomyes cerevisiae.* Extract in petroleum ether was active against the three organisims : (1)E. coli (2) *Rhizobium* sp. and (3) *S. cerevisiae.* Alcohol extract was active against the three organisms: (1) *E coli* (2) *Rhizobium* sp. and (3) *S. cerevisiae.* Chloroform extract of leaf and hair contact extract was active against the two micro organisms: *E, coli* and *Rhizobium* sp.. No extract showed any activity against *Aspergillus* sp.. Control in distilled water has no activity against any of the five microaganisms tested.

Aqueous extract of the poisonous plant was not found to be active against *Rhizobium* sp. *Agrobacteria* sp. and *Aspergillus* sp.. Sap extract

of leaf was not active against *Aspergillus* sp.. Extract in petroleum ether was not active against *Agrobacteria* sp. and *Aspergillus* sp.. Extract in alcohol was not active against *Agrobacteria* sp. and *Aspergllus* sp.. Extract in chloroform and hair contact extract has no activity against *Agrobacteria* sp. *Saccharomyces cerevisiae* and *Aspergillus* sp.. .

The highest zone of inhibition (10.22mm) was recorded with leaf sap extract against *E. coli* using YEP medium similar antimicrobial activities of different plant parts have been reported previously (Sokomba *et al.* 1986, Hassan *et al.* 1988, 1989, Hossin *et al.* 1993, Khan *et al.* 1989, Chowdhury *et al.* 1988, Hoque *et al.* 1989, *Mia et al.* 1997, Shafiqur *et al.* 1997, Alam et *al.* 1992, *Datta et al.* 1992, Howlader *et al.* 1992, Mannan *et al* 1996).

Table 3: Studies of Antibacterial Activities of *Laportea Crenulata* Plant Extracts on Yep Medium.

Test Organisms	Disc No.	CL Dist Wt. 5/plate	AQ. Ext. Discs 6/plate	Leaf sap Ext. Discs 6/plate	Leaf Ext. in PE Discs 6/plate	Leaf Ext in Ale. Discs 6/plate	Leaf Ext. in CF. Discs 6/plate	Hair cont. of Discs 5/plate
Rkizobium sp.	1	0	0	10	12	11	10	7
	2	0	0	10	9	10	9	7
	3	0	0	8	10	9	9	8
	4	0	0	7	8	9	7	8
	5	0	0	10	8	7	10	7
	6		0	8	9	7	7	
	7							
AV. area inb. zone		0	0	8.83	9.33	8.83	8.66	7.4
Agrobacteria sp.	1	0	0	9	0	0	0	0
	2	0	0	9	0	0	0	0
	3	0	0	7	0	0	0	0
	4	0	0	8	0	0	0	0
	5	0	0	7	0	0	0	0
	6	0	0					
	7	0						
AV. area inb. zone		0	0	8	0	0	0	0

Table 4: Studies of Antifungal Activities of *Laportea Crenulata* Plant Extracts on Yep Medium.

Test Organisms	Disc No.	CLDist Wt. Discs 5 /plate	Diameter of Inhibition Zone in Milimeter (mm) after 48 Hrs of Incubation					
			AQ. Ext. Discs 5 /plate	Leaf sap Ext. Discs 5 /plate	Leaf Ext in PE Discs 5 /plate	Leaf Ext in Alc. Discs 5 /plate	Leaf Ext. in CF. Discs 5 /plate	Hair cont. of Discs 5 /plate
S. cerevisiae	1	0	10	10	8	12	0	0
	2	0	10	8	9	12	0	0
	3	0	8	10	12	8	0	0
	4	0	8	8	10	10	0	0
	5	0	7	8	9	8	0	0
	6							
	7							
AV. area inb. zone		0	8.6	8.8	9.60	10	0	0
Aspergillus sp.	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
	6							
	7							
AV. area inb. zone		0	0	0	0	0	0	0

Table 4: Effect of Different Media. Average Zone of Inhibition in Diameter (mm) Using Three Medium against Organisms Tested.

Media	Test organisms				
	Escherichia coli	*Rhizobium sp.*	*Agrobacteria sp.*	*S. cerevisiae.*	*Average zone of inhibition*
YEP	9.08	8.61	8.00	9.25	8.73
YEM	8.69	8.45	8.16	9.05	8.58
NA	8.39	8.26	7.83	8.90	8.34
Average zone of inhibition	8.72	8.44	7.99	9.06	

Aswal *et al.* (1984) did antibacterial tests with alcoholic extract of *P. hydropiper* sp. Microcarpum against *Strep faecalis* and *Klebsiella puenmoniae* and carried out the some tests with alcoholic extract of *P. Chinense* L. Var ovalifolia Meisn. against *B. subtilis,* staph, aureus, *S. typhi, E. coli* and *Agrobacterium tumefaciens* and they found no antibacterial activity. In the present study alcoholic extract *ofLaportea crenulata* showed similarity with their studies.

The inhibition effect as found in the plant extracts *ofLaportea crenulata* is of great importance. This activity against a wide range of bacteria and fungi the inhibitory substance (s) of the plant extracts has been found in this study, may be used for the therapeutical purposes of animals and human being after studying their toxicity effect in detail.

I think this research report will open a new field of research in Oncology and Tumour. We are hopeful to isolate the active ingredients and to test against malignant cells.

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