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Pharmacological Investigation of Ethanolic Extract of Roots of Holoptelea Integrifolia (Roxb.)Plench

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Abstract: ***The present study deals with pharmacological studies of ethanolic extract of roots of H. integrifolia (Roxb.) Planch (Family: Ulmaceae) . The ethanolic extracts of roots were examined for antimicrobial studies by using Disc diffusion method , antiviral efficacy was undertaken by using plaque inhibition method and antineoplastic activity was tested by using Sarcoma 180A^0 as the test system . The data of present study suggested that H. integrifolia root extract showed pronounced activity against test bacteria and viruses. It also exhibited antifungal efficacy but failed to demonstrated any antitumor activity.***

Keywords : ethanolic extract, roots , antimicrobial activity, antiviral activity, antitumor activity

I. INTRODUCTION

The plant *Holoptelea integrifolia* (Roxb.) Planch.belongs to the family Ulmaceae. It is commonly known as "Chilbil". *H. integrifolia* is a large spreading glabrous deciduous roadside tree distributed throughout the country up to an altitude of 600 m^3. Only few plants of the family Ulmaceae are known, which have medicinal importance[1,2] like fruit of Celtisaustralis (*syn. C. caucusida*) is used in amenorrhoea and colic, *C. cinamomea, C. wightii* and *C. reticulata* (syn. *Gironnierareticulata*) are used as a blood purifier in itch and other cutaneous eruptions[1]. A review of literature revealed that *H. integrifolia* is also medicinally important ,the juice of boiled mucilaginous bark is applied to rheumatic swellings, the stem fibers tied to the upper arm are useful in patients suffering from malarial fever[1-4] the crude leaf sap of *H. integrifolia* was found to be mildly active against bean common mosaic virus[5] but no biological studies has been done on roots of this plant so far. Hence a systematic pharmacological examination of roots of this plant were undertaken.

II. MATERIALS AND METHODS

The roots of Holoptelea integrifolia (Roxb.)Planch. Were collected from the Rajasthan University Campus, Jaipur and identified from the Department of Botany, University of Rajasthan, Jaipur (Herbarium sheet No. RUBL 4334). The roots were dried under shade,then powdered with a mechanical grinder and stored in airtight container.

A. Bactericidal and Fungicidal Assay

Ethanolic extract of the powdered roots was prepared on a steam bath for 8 X 3 hrs. The extract was concentrated under reduced pressure and stored in dark colored bottle at 4^{0}C in a refrigerator. For both bactericidal and fungicidal assays Disc diffusion method[6] was adopted , because of re-productivity and precision. The different test organisms (Bacteria Pure culture of all *Escherichia coli, Klebsiella aerogenes, Proteus* vulgaris and *Salmonella paratyphi* B as Gram -ve and *Staphylococcus aureus* as Gram +ve bacteria,

1) *Fungi: Aspergillus flavus, Aspergillus niger, Fusarium moniliforme* and *Rhizoctonia bataticola*) were preceded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whatman No. 1 paper (6 mm in diameter) , which were containing 500 µg and 1000 µg of the test extracts and control amikacin (10 µg/ml for bacteria) and nystatin (100 units /ml for fungi) as reference drugs separately. These plates were initially placed at low temperature for 1 h , so as to allow the maximum diffusion of the compound from the discs into the agar plate and later, incubated at 37^{0}C for 24 hrs in case of bacteria and 48 hrs for fungi, after which the zones of inhibition could be easily observed.

B. Virucidal Efficacy

50g of the fresh plant material (roots) were macerated in a waring blender with 150 ml of 80 % ethanol. The mixture was stirred for 2hrs. at room temperature , filtered (E1) and marc was percolated (E2) with 80% ethanol again to exhaustion. Both of filtrate (E1) and percolate (E2) were pooled and concentrated to a thick residue at a temperature, not to exceed 40^{0}C. Each of the residual mass was dissolved separately in sterile 0.01 M physiological tris buffer (8ml;pH7.2) diluted with tissue culture medium (M-2; 128 ml) and pH adjusted to 7.2 by using 1N NaOH. The diluted extract was filtered through Whatman GF-2 paper disc followed by

sterilization (by filtration through Millipore membrane filter,0.02 µ) and later , stored at ~ 30^0C, until tested.

The virucidal activity of root extract which was obtained in the virucidal efficacy tests was always confirmed by Plaque inhibition method[7].Confluent cultures of VERO cells were prepared in tissue culture dishes (50 mm diameter) and infected with 0.2 ml Poliomyelitis virus suspension in M-2 medium containing approx. 100PFU[8]. After an absorption period of 1 hr , the cells were washed to remove any unabsorbed virus and overlaid with 5 ml tissue culture medium (M-2) and 0.8% acetone purified agar at 420C. After the solidification , a paper disc (6 mm diameter) impregnated with 0.2 ml of serial 2-fold dilution of plant extracts in M-2 medium was centrally placed on the surface of agar overlay and incubated at 370C for 2 days. A second agar overlay of the same composition supplement with 0.03% neutral red was added and incubation was continued for at least 2 days at 370C until the plaques were well developed. The discs were surrounded by a clear zone of toxic cells destruction and outside this, by plaque –free zone, where the zone of plaques were smaller than the normal, and later, the plaque-inhibition zones were measured in mm.

C. Antineoplastic Efficacy

100g each of dried and powered plant material (roots) were percolated at room temp. for 48 h and the remaining residue was re extracted using soxhlet apparatus for 16 h . Both these fractions were pooled together, dried under reduced pressure and stored at room temp. , until used.

Total packed cell volume method was adopted for the antineoplastic screening[9]. Five weeks old ICR albino mice (20± 3 g) implanted i.p. Sarcoma - 180Å (1x 10^6 cells/0.1 ml ascitic fluid) was used for experimentation in each of the mice. In each experiment, six animals per test group were used. Each of the test sample suspended in physiological saline solution mixed with 0.5 % carboxy-methyl-cellulose (CMC) was given daily to the test animals at a dose of 100mg/kg/day(i.p.) for consecutive 5 days. A control set was also run parallel ,where the animals were given only physiological saline solution . Each of the experimental animals were sacrificed after 7 days from ascites tumor implantation. The ascites of each animal was centrifuged (3000 rmp) for 5 min to isolate the tumor cells, the volume of the tumor cells (packed cell volume ; PCV) and total volume of the ascites (total volume ; TV) were determined in each case. Besides this, body weight change (BWC) was also recorded by determining the difference in the weight after 7 days of ascites tumor implantation and the weight of the tumor implanted. Growth ratio (GR) was calculated (%) as the ratio between the average of PCV of control group and of the treated group.

III. RESULTS AND DISCUSSION

In case of bactericidal activity against *Escherichia coli, Klebsiella aerogenes, Proteus* vulgaris, *Salmonella paratyphi* B and *Staphylococcus aureus* ,the ethanolic extract of roots showed pronounced activity against *S. paratyphi* B at 1000 µg/disc , in this case the activity was equal to that of standard (AI= 1:00), it also exhibited appreciated activity against E.coli at 1000 µg/disc. As shown in Table 1

In case of antifungal activity against *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme* and *Rhizoctonia bataticola* , the extract of roots demonstrated maximum inhibition against *A. fiavus* (AI=0.78,1000 µg/disc) and significant activity (AI=0.60,1000 µg/disc) against *F. moniliforme*. As shown in Table 1

Table 1. Bactericidal and fungicidal efficacy of roots of *Holoptelea integrifolia.*

		Test Bacteria					Test Fungi			
		E.coli	*K.aerogenes*	*P.vulgaris*	*S.paratyphi B*	*S.aureus*	*A.flavus*	*A.niger*	*F.moniliforme*	*R.bataticola*
Dose										
1000µg/disc	IZ	13	-	-	18	-	15	10	17	14
	AI	0.39	-	-	1.00	-	0.78	0.41	0.60	0.53
500µg/disc	IZ	-	-	-	±	-	11	8	15	12
	AI	-	-	-	-	-	0.57	0.33	0.53	0.46

IZ inhibition zone (in mm) including the diameter of disc (6mm)

AI activity index=(inhibition zone of sample/inhibition zone of standerd

Standerd: Amikacin = 10 µg/ml (becteria) ; Nystatin = 100 units/disc (fungi)

(±) Trace activity: (-) No activity

In case of antiviral activity against the test viruses – *Semliki forest, Herpes simplex, Measles* and *Vesicular stomatitis* , The ethanolic extract of roots of *H. integrifolia* demonstrated pronounced activity against *Semliki forest* (R = 10^5, 250 µg/ml; R = '10^4, 125 µg/ml) and *Herpes simplex* (R = 10^4, 250 µg/ml; R = 10^3, 125 µg/ ml) but, weak activity against *Poliomyelitis* (R = 10, 125 µg/ml). As shown in Table 2

Table 2. Virucidal efficacy

Dose	Antiviral Activity					
	poliomyelitis	coxsackie	semliki forest	herpes simplex	measles	vesicular stomatitis
500µg/ml	NT	T	T	T	T	1
250µg/ml	NT	1	10^5	10^4	-	-
125µg/ml	10	1	10^4	10^3	1	1
50µg/ml	1	1	10	10	1	1

Table 3. Antitumor activity

Part used	Dose (mg/kg/day)	Toxic death	BWC[a]	PCV[b]	GR[c]	Judgement
roots	100	0	-1.7	0.29	100.0	-

c = Growth ratio =0-10% +++ ; 11-40% ++ ; 41-65% + ; 66% onwards - .

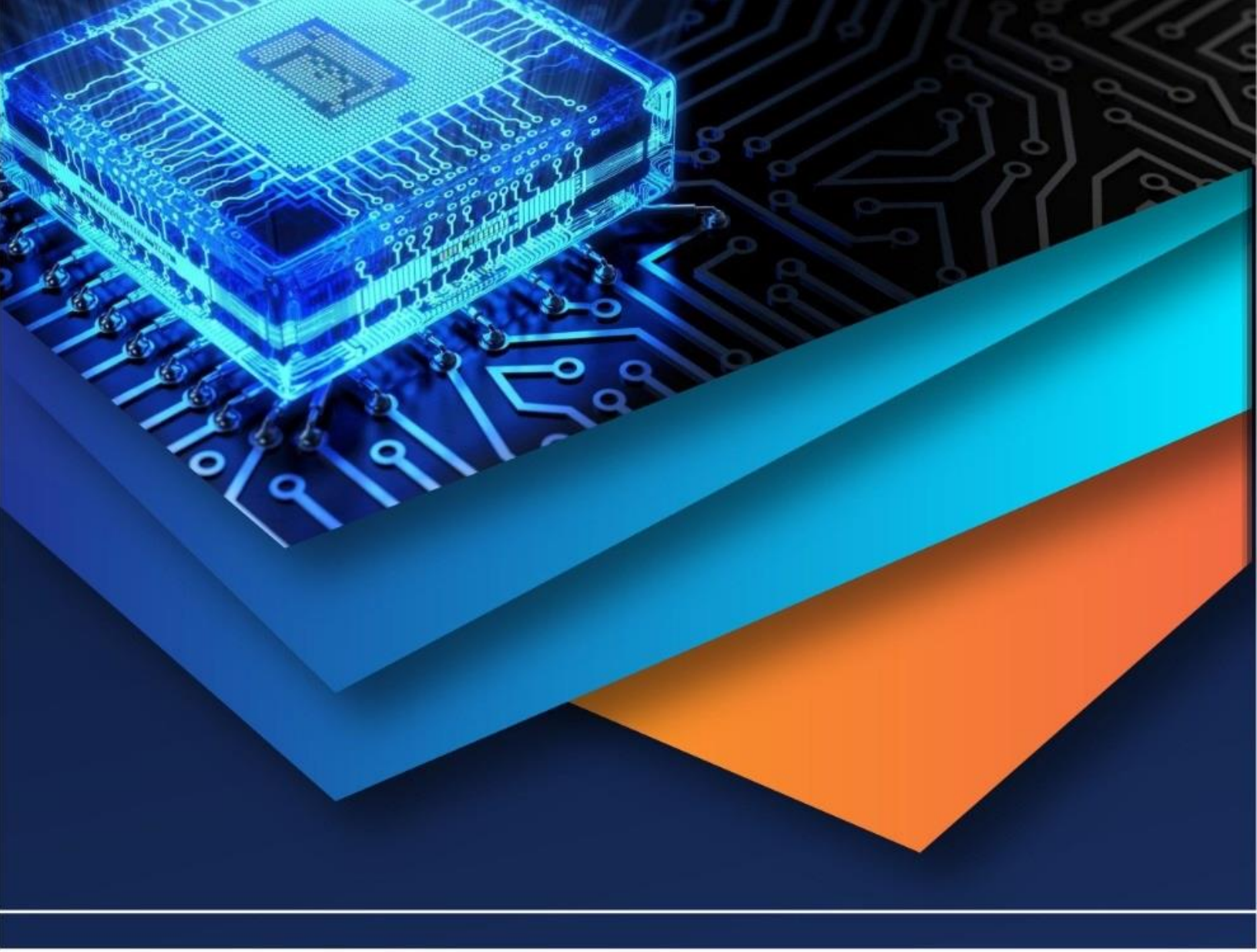
In case of antitumor activity , the root extract of plant failed to exhibit any activity using Sarcoma-180 ascites in mice (Total Packed Cell Volume method). . As shown in Table 3.

IV. CONCLUSION

On the basis of above activity results we can say that the roots of H. integrifolia exhibited pronounced antibacterial and aniviral activity . It also exhibit good antifungal activity but does not show any antitumor activity.

REFERENCES

[1] R.N.Chopra , S.L. Nayar and I.C.Chopra , “Glossary of Indian medicinal plants” ,CSIR,New Delhi, 1971.
[2] K.R. Kirtikar and B.D. Basu ,” Indian medicinal plants” .Lalit Mohan Basu and Co., Allahabad, vol.III, p2292, 1975.
[3] ICMR, Medicinal plant of India ,New delhi, vol II, p48,1987.
[4] K.Hemadri ,P.V. Raj , S.B. Rao and C.R.R. Sharma , J. Sci Res,Pl.Med.,vol I(2),p37,1980
[5] R.N. Tripathi, R.K.A. Tripathi and D.K. Pandey, Environment India, vol **4**,p 86 ,1981.
[6] J.C. Gould and J.H. Bowie, Edinb. Med. J., vol **59**, p178,1952
[7] R. Dulbacco, Proc. Nat. Acad. Sci. Wash., vol **38**, p747,1952
[8] V. Hronovsky, U. Plaisner and R. Benda, ActaViral.,vol **19**,p150, 1975.
[9] H. Itokawa, K. Watanabe and S. Mihashi, ShoyakugakuZassh,vol **33** ,p 95, 1979.



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