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Antimicrobial Activity of Saponins Isolated from *Achyranthus aspara* against *Staphylococcus aureus*

SUNITA SHARMA†, P.N. SHRIVASTAVA† and R.C. SAXENA*
Pest Control and Ayurvedic Drug Research Laboratory
S.S.L. Jain Postgraduate College, Vidisha-464 001, India
E-mail : rcsvds@yahoo.com

Achyranthus aspara Linn. (family Amaranthaceae) alcoholic extract showed the presence of the triterpenoid saponin with dose dependent inhibitory activity against *Staphylococcus aureus*, a bacteria causing skin disease in human beings. Minimum inhibitory concentration was found to be highest (0.15 mg) for purified fraction-3. The identification of the compound on spectral analysis gave a triterpenoidal saponin purified fraction.

Key Words: Minimum inhibitory concentration, *Staphylococcus aureus*, Antimicrobial, Saponins.

INTRODUCTION

Plants possess several secondary metabolites as the chemicals for their defense. Saponins having various pharmacological effects are widely distributed in plants. It is reported that saponins are used for their antibacterial[1], antifungal[2], antiviral[3], cytotoxic, immunomoduatory[4] and antihistaminic[5] activities.

Abbasoglu and Turkoz[6] have reported the antimicrobial activities of saponin extract from some indigenous plants of Turkey. The essential oils of several plants of family Rutaceae, Lamiaceae and Asteraceae have been reported by various workers to possess antimicrobial activity[7]. Ganjalez *et al.*[8] and Parmeshwari and Latha[9] have reported the various solvent extracts of *Riccinus communis* leaves against five species of bacteria and have found positive antibacterial effect. Koli *et al.*[10] have also reported antimicrobial activity of isoflavone glycoside.

Looking to the pertaining literature and in view of the necessity to search the suitable antibacterial drugs from plants, the present investigation was proposed to be investigated against *Staphylococcus aureus*, a causative agent of skin diseases.

†Division of Microbiology and Plant Pathology, Department of Botany, S.S.L. Jain P.G. College, Vidisha-464 001, India.

EXPERIMENTAL

Achyranthus aspara Linn. of family Amaranthaceae called Apamarg was collected from the local sorroundings of Vidisha (M.P.). After proper identification in the Botany Department, a voucher specimen was procured in the herbarium record of Pest Control and Ayurvedic Drug Research Lab. at Vidisha (M.P.) India at S.No. 17.

Preparation of extract: The dried whole plant powered material was extracted overnight with 70% ethanol by cold percolation method as well as soxhlet apparatus. Thereafter the material was repeatedly extracted with hot aqueous ethanol for three times. The soluble part was concentrated over a boiling water bath. The concentrate crude drug gave 2.65% yield. The ethanolic and water extracts were used for the present study as shown in Table-1.

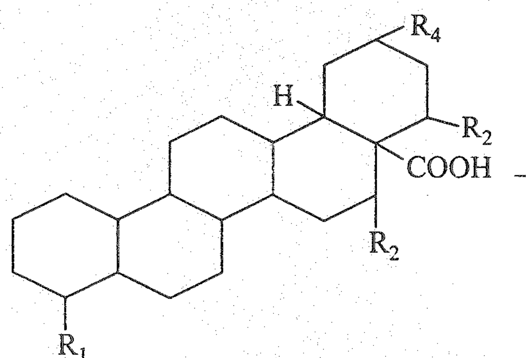
Chemical analysis of crude drug: The biologically active compound was separated from the crude extract by column chromatography[11]. It was followed by TLC using $CHCl_3 : MeOH : H_2O$ (2 : 2 : 1) and $CHCl_3 : CCl_4$: acetone (6 : 6 : 3) and $CHCl_3 : MeOH : H_2O$ (2 : 4 : 2). This gave three fractions. Each fraction was further chromatographed till a single spot was obtained as shown in Table-2.

Acid hydrolysis of purified fraction: The compound was hydrolyzed with 10% H_2SO_4 in $MeOH : H_2O$ (1 : 1) at 800°C for 4 h. The reaction mixture was neutralized with $BaCO_3$ and filtered. The filtrate was evaporated to dryness in vacuum to give a residue in which glucose was identified.

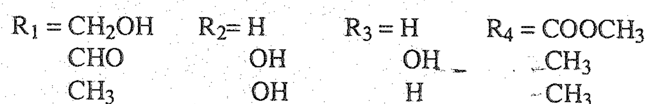
Methylation: The acid hydrolyzed fractions were methylated after the removal of methanol; the solution was extracted with ethyl acetate three times. The extract was crystallized with MeOH repeatedly.

Glycosidal test of the fraction: The four fractions were tested chemically for glycosides which show the presence of mono and disaccharides in the compound. Iodine and Benedict's tests were also used to confirm the presence of glycosides.

Spectral analysis: The spectral analysis of the compound was carried out for NMR which showed no signals for an aldehylde group at C-23. Instead, signals for two carbonyl carbon (δ 177.6 and 178.9) are present in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed resonance for only five methyl groups on tertiary carbon at δ 0.73, 0.81, 0.98, 1.14 and 1.19 (s, 3H each). A three proton singlet was shown at δ 3.73 with a one bond heteronuclear correlation to the carbon resonance at δ 52.3 and a correlation to the carbonyl signals at δ 178.9. Additionally an oxymethylenic group (δ 3.75) was also shown. The compound has the highest molecular mass of the saponin isolated from 70% ethanol. The R_f value of the compound was found between 0.3 to 0.5 which indicates the presence of saponin. On the basis of this, a triterpenoid saponin was isolated from the whole plant of *Achyranthus aspara*.



Triterpenoid saponin



$R_1 = CH_2OH$	$R_2 = H$	$R_3 = H$	$R_4 = COOCH_3$
CHO	OH	OH	CH_3
CH_3	OH	H	CH_3

Microbial study: *Staphylococcus aereus* ATCC 25923 bacterial strain was used for the present study. The bacterial strain was procured from NIV, Pune.

Inoculation suspension: The microorganism suspensions used for inoculation was prepared at 10^6 cfu/mL by diluting fresh culture.

Media: Agar liquid nutrient medium was used for diluting the microorganism suspension and for twofold dilution of the extract.

Method: Microdilution method was employed for antibacterial tests[12]. Dried extract was prepared with distilled water at a concentration of 5%. The solution of each extract (2.5, 1.25, 0.63%) was prepared in microplates by diluting media.

Suspensions of microorganism were added to the extract to yield concentration of approximately 5×10^4 cfu/mL. Distilled water microorganism mixture, microorganism and media were used as control.

Since turbidity was observed with extracts during dilution with liquid medium, it was difficult to determine the growth of the cultures macroscopically. Therefore, each dilution of the series was inoculated on dextrose agar and Hinton agar with a loop after adding the culture. All the petri dishes were inoculated at 36°C for 16–20 h. After this period, colonies were visible. The concentration of extract where no growth was seen was identified as the minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Table-1 reports the percentage yield of crude drug in soxhlet at different solvents including 90% methanol and water. Maximum yield was noticed in water extract. This shows that *Achyranthus aspara* contains more polar compounds than non-polars. Table-2 shows the TLC fractions of the crude drug obtained from the plant along with their R_f value which when compared with the R_f value of authentic marker compounds (Sigma Aldrich Co. Pvt. Ltd, USA) shows the presence of saponin. The different concentrations of the purified fractions were applied on the petri dishes containing bacterial culture. After 24 h, inhibition of

growth was measured and maximum inhibitory concentration was worked out as shown in Table-3.

TABLE -1
PERCENTAGE YIELD OF *ACHYRANTHUS ASPARA* BY SOXHLET EXTRACTION AT 40°C

Solvent used	Wt. of powder (g)	Wt. of extract (g)	Yield of crude drug (%)
n-Hexane	260	5.62	2.620
Chloroform	700	6.42	3.210
Ethyl acetate	200	2.85	1.425
70% Methanol*	200	5.30	2.650
Water*	200	15.23	7.660

* Both the solutions gave polar compounds.

TABLE-2
TLC OF CRUDE DRUG OF *ACHYRANTHUS ASPARA*

Solvent system	Fraction	Spot No.	Behaviour		R_f value of each spot
			Visible	UV light	
$CHCl_3 : MeOH : H_2O$ (2 : 2 : 1)	A1	Spot I	Dark green	Dark green yellow	0.31
$CHCl_3 : CCl_4 : H_2O$ (6 : 6 : 3)	A2	Spot 1	Light green	Brown	
		Spot 2	Light yellow	Green	0.42
		Spot 3	Dark green		
$CHCl_3 : MeOH : H_2O$ (2 : 4 : 2)	A3	Spot 1	Light brown	Dark brown	0.56
		Spot 2	Yellow brown	Yellow	

The MIC values of the extracts are shown in Table-3 which shows dose dependent inhibition of bacterial growth.

TABLE-3
MIC VALUE OF THE DIFFERENT FRACTIONS OF SAPONIN OF *ACHYRANTHUS ASPARA* AGAINST *STAPHYLOCOCCUS AUREUS*

Saponin fraction	Microorganism minimum inhibitory concentration
Fraction 1	0.63
Fraction 2	0.31
Fraction 3	0.15

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Contact:
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Tel: (973)(17)710868
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Web: http://www.green-industry.org/